University of Alberta

Enamel trace element composition and palaeodietary studies - an exploratory model

by

Caroline Maria Haverkort ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Department of Anthropology

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *Enamel trace element composition and palaeodietary studies – an exploratory model* submitted by Caroline Maria Haverkort in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Rhonda C Bell
Rhonda Bell

Pamela Willoughby for
Gisela Grupe

Michael Wayman

Date approved: 25th September 2001

This work is dedicated to the memory of
Alberto Musacchio

तदा द्रष्टुः स्वरूपेऽवस्थानम्।

Tadā draṣhṭuḥ svarūpe’vasthānam

> “When thought ceases,
> the spirit stands in its true identity
> as observer to the world”
>
> Sutra 1.3

From: *Yoga - Discipline of Freedom*
The Yoga Sutra attributed to Patanjali
Translated by Barbara Stoler Miller (1998)

ABSTRACT

Teeth are increasingly being used in palaeodietary studies. Therefore, the objective of this study was to explore the research potential of enamel trace element composition in relation to infant and child nutrition, including weaning, an important palaeodemographic variable.

For palaeodietary studies, we need to understand when and how trace elements are built into enamel, and to what extent the trace element concentrations in enamel reflect the trace element concentrations in consumed foods and water. Both maternal diet and, after weaning, the child's diet, play a role in determining enamel composition for the deciduous and permanent dentition.

The study includes experiments with regard to sample preparation methods and analytical techniques (including neutron activation analysis, electron microprobe and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)), applied to both archaeological and modern tooth samples.

Dental and nutritional literature is used to develop a theoretical model which describes the trace element pathways between mother and fetus or infant, in relation to the different stages of dental development. Predictions based on this theoretical model, and on traditional palaeodietary methods, are compared with the enamel trace element composition of teeth from five individuals of known dietary intake, as determined by LA-ICP-MS. With this technique, concentration differences between and within teeth could be demonstrated. The individuals could be clearly separated using strontium and barium concentrations. However, a direct relationship between enamel trace element composition and known dietary intake can not be demonstrated in this study, partly due to technical difficulties inherent in the use of this new analytical technique. Therefore, at this stage, a more detailed evaluation of the relative merits of the traditional palaeodietary methods and the model developed in this study, is not possible.

The results suggest that LA-ICP-MS is a very promising microanalytical technique, yielding high resolution data which can be correlated with stage of dental development. Suggestions are offered to improve sample preparation methods and analytical procedures. It is argued that it is essential to develop and optimize the methodology on samples of known dietary history, prior to application of this technique to archaeological samples for palaeodietary reconstructions.

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LIST OF ABBREVIATIONS

AAS: atomic absorption spectrometry
ATP: adenosine triphosphate
BL: bucco-lingual
CEJ: cementum enamel junction
EDJ: enamel dentine junction
EDS: energy dispersive spectrometer
ENAA: epithermal neutron activation analysis
EPMA: electron probe micro analysis
ICP-AES: inductively coupled plasma atomic emission spectroscopy
ICP-MS: inductively coupled plasma mass spectrometry
IMS: industrial methylated spirit
INAA: instrumental neutron activation analysis
LA-ICP-MS: laser ablation inductively coupled plasma mass spectrometry
LEH: linear enamel hypoplasia
MD: mesio-distal
NAA: neutron activation analysis
NIST: National Institute of Standards and Technology
REEs: rare earth elements
RSD: relative standard deviation
SEM: scanning electron microscopy
SIMS: secondary ion mass spectrometry
TRA: time resolved analysis
WDS: wavelength dispersive spectrometer
XRF: x-ray fluorescence

Deciduous dentition:

di1	central incisor
di2	lateral incisor
dc	canine
dm1	first molar
dm2	second molar

Permanent dentition:

I1	central incisor
I2	lateral incisor
C	canine
P3	first premolar
P4	second premolar
M1	first molar
M2	second molar
M3	third molar

NB: Throughout this thesis, lower case will be used to indicate deciduous teeth, and upper case will be used to indicate permanent teeth.

CHAPTER 1

INTRODUCTION

Reconstructions of subsistence and diet

The study of prehistoric diet and subsistence has always played a major role in archaeological research. The subsistence system provides insights into a population's adaptation to its environment. Patterns of morbidity and mortality, which may be related to dietary adequacy, can tell us something about the success of this adaptation. In addition, various social groupings, based on cultural and biological variables, such as age, sex, or status, can be expressed through differential access to food. The change in subsistence and dietary patterns that accompanied the development of agriculture in various parts of the world is intricately linked to social and cultural changes, and is fundamental to our understanding of the development of more complex societies.

The traditional methods for the reconstruction of subsistence systems are based on a qualitative and quantitative analysis of faunal and floral remains recovered from a site, in conjunction with environmental reconstructions and information from associated tools and weapons (Wing & Brown, 1979). Such analyses enable us to make general statements regarding the resources available to a population. They do not, however, provide insights into the actual food intake (diet) of the people involved, or differences in individual food consumption. An additional drawback is that organic remains are not always preserved, which will leave many types of (potential) foods undetected.

During the last few decades, the study of prehistoric diet and subsistence has been revolutionized with techniques for the chemical analysis of human remains: trace element and stable isotope analysis (Price, 1989). These methods are based on a number of assumptions, the most fundamental one being that the chemical signature of skeletal hard tissues (bones and teeth) will reflect the chemical signatures of the foods consumed. An important advantage offered by the chemical methods, which involve sampling individual skeletons, is the potential for inferring consumption patterns associated with social groupings (based on sex, age, status, for example), either between or within populations.

In trace element analysis, which is based on the inorganic hydroxyapatite component of bone, certain trace elements, e.g., copper (Cu)[1] and zinc (Zn) have been used as indicators of meat consumption, whereas other elements such as manganese (Mn) and strontium (Sr) have been used as indicators of the vegetable component of the diet. Other researchers have used trace elements, in particular barium (Ba) and Sr, to distinguish between marine-oriented and terrestrial-oriented subsistence systems. For reviews of the trace element analysis of bone and teeth see e.g., Sandford (1992, 1993) and Sandford & Weaver (2000).

In stable isotope studies, most applications use the collagen fraction of bone to determine the carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios. Stable isotopes are used to distinguish between foods from different types of habitat. The basic principle is that the isotope ratios vary due to fractionation processes along the food chain. Carbon isotopes can be used to distinguish between plants using different photosynthetic pathways (mainly C_3 and C_4). This approach has made important contributions to, for example, our understanding of the process of the introduction of maize, a C_4-plant, as opposed to most plants of the temperate zone, which use the C_3-system. N-isotopes are primarily used to estimate the relative contribution of marine and terrestrial foods to the diet. For reviews see e.g., Keegan (1989), Katzenberg (1992, 2000) and Ambrose (1993).

Although both trace element and stable isotope analyses offer several advantages over the traditional methods, it is important to point out that the chemical methods can only be applied within the context of information provided by the traditional methods. In addition, the two different chemical approaches focus on somewhat different aspects of diet and subsistence, and in that sense they are complementary. The three major research objectives of palaeodietary studies according to Sandford (1992) are:

- Determination of relative proportions of different resources (vegetable vs. meat; marine vs. terrestrial - e.g.: Schoeninger & Peebles, 1981; Schoeninger *et al.*,

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[1] Throughout this thesis, the trace elements that are discussed are named in full at first use, together with their chemical symbol. Thereafter, only the symbol will be used. For easy reference, a periodic table is included in Appendix A.

1983; Connor & Slaughter, 1984; Burton & Price, 1990; Harritt & Radosevich, 1992; Subirà & Malgosa, 1992);

- Socio-cultural correlates of dietary intake (e.g., Schoeninger, 1979; Blakely & Beck, 1981; Geidel, 1982; Pérez-Pérez & Fox, 1992);
- Diachronic dietary change, e.g., the shift from hunting-gathering to farming (e.g., Schoeninger, 1981; Tauber, 1981; Katzenberg *et al.*, 1995).

A comparison of bone and teeth

Since the development of chemical analysis for palaeodietary studies, bone has been the most frequently used sample material. Recently, however, there has been an increase in interest in the use of teeth. Although there are several similarities between the two tissues, there are also some important differences, which will be discussed below.

Bone and teeth both consist of an inorganic phase of hydroxyapatite crystals and an organic phase of proteins and water. During bone formation the bone forming cells (osteoblasts) excrete a matrix, which is subsequently mineralized by hydroxyapatite crystals. These crystals are *embedded in* the organic matrix, largely consisting of collagen. In contrast, during the formation of dental enamel, the enamel forming cells (ameloblasts) initially deposit a matrix of tooth-specific proteins, mainly enamelins and amelogenins, which is only partially mineralized. During the subsequent maturation stage, these proteins are withdrawn from the maturing enamel on a massive scale to make room for the growing hydroxyapatite crystals. Thus, in enamel, the crystals *displace* the organic phase during mineralization.

Mature enamel is mineralized to a very high degree (about 97-98%); in fact, it is the most highly mineralized biological tissue known (Aiello & Dean, 1990). Bone, on the other hand, is only about 65% mineral and remains a living tissue throughout life. Hydroxyapatite can be described with the basic formula $Ca_{10}(PO_4)_6 \cdot (OH)_2$. However, various trace elements can substitute for the main constituents of the crystal lattice. For example, Sr^{2+} can replace calcium (Ca^{2+}) and fluoride (F^-) can substitute for OH^-. The hydroxyapatite crystallites in bone are almost ten times smaller in all dimensions than the crystallites in enamel (Jenkins, 1978; Simmer & Fincham, 1995). This gives bone apatite a higher surface-to-volume ratio, which accounts for its greater propensity to ionic exchange when compared to enamel. This exchange capacity is the basis for one of the metabolic functions of bone, maintaining mineral homeostasis. However, the exchange of ions between apatite and its environment is not limited to life but continues after death (the '*biogenic-diagenetic continuum*' – Sandford, 1993), with obvious implications for bone chemistry studies. The term *diagenesis* is used to refer to post-mortem chemical alterations in skeletal remains. The soil pH, water content, microorganisms, temperature (including freeze-thaw cycles) and size of bone all affect diagenetic processes. Depending on the local conditions, diagenetic changes may range from "minor changes in the bone protein to complete structural and chemical breakdown" (Von Endt & Ortner, 1984: 247). Because of its larger crystallites and high degree of mineralization, enamel is much less susceptible to such chemical changes than bone (Kyle, 1986; Bell *et al.*, 1991; Ericson, 1993).

During life, bone is subject to constant remodelling – the absorption and deposition of bone by osteoblasts and osteoclasts (Sandford, 1993). During remodelling, new bone will be formed from nutrients supplied by the blood, the composition of which is a function of the individual's nutritional status. Because of a relatively slow turnover of bone material, the chemical composition of bone is considered representative of dietary intake and environment during the last 7-10 years of an individual's life. In contrast, once enamel formation and mineralization have been completed, the ameloblasts are lost and the tissue remains unaltered, apart from the outermost layer which is capable of taking up ions from the oral environment (Cutress, 1983). Therefore, chemical composition of enamel reflects dietary intake and environment during the period of tissue formation, which for the deciduous dentition is the period *in utero* and infancy, and for the permanent dentition encompasses infancy and childhood.

One of the interesting characteristics of teeth is that the different tooth types develop at specific developmental ages, in a reasonably predictable order. This means that the composition of different teeth, for example the first permanent molar (M1) and the second permanent molar (M2), represent dietary intake during the period from birth until about 3 years, and from about 3-7 years of age, respectively (Hillson, 1996). The timing of specific dietary changes, provided these changes 'leave' a chemical signature, can in this way be traced to relatively narrow age ranges. These principles have been applied in studies of weaning age in prehistoric populations (e.g., Katzenberg *et al.*, 1996; Schurr, 1997, 1998; Herring *et al.*, 1998; Wright & Schwarcz, 1998, 1999). An even more intriguing possibility is that the incremental growth lines in enamel, which arise during the rhythmic deposition of enamel matrix at the time of formation, would allow us to determine with even more accuracy the timing of dietary changes or, for example, the occurrence of dietary stress.

Trace elements and stable isotopes

After the first pioneering studies (Brown, 1973; Gilbert, 1975; Vogel & Van der Merwe, 1977; DeNiro & Epstein, 1978, 1981), researchers were quick to appreciate the enormous potential for applications of the chemical methods to archaeological problems. However, the adoption of the techniques was soon followed by the recognition of several difficulties. One of the major problems for trace element studies was diagenesis. The possibility, or even likelihood, that analysis of bone samples provided a diagenetic, i.e., *non-biogenic*, signal dampened the initial enthusiasm and led to a wave of scepticism (see e.g., Buikstra *et al.*, 1989; Sillen *et al.*, 1989; Radosevich, 1993; Ezzo, 1994a, b). On a more positive note, the realization that bone chemistry research was going to be more complicated than anticipated prompted research in a variety of directions, for example:

- the extent and nature of diagenetic alteration in bone under varying burial circumstances (see e.g., Lambert and co-workers: 1979, 1983, 1984, 1985, 1989; Gordon & Buikstra, 1981; White & Hannus, 1983; Grupe & Piepenbrink, 1988);
- modelling of diagenetic processes (Child, 1995; Hedges & Millard, 1995; Hedges *et al.*, 1995);
- development of methods to detect or control for diagenetic effects (Lambert *et al.*, 1984; Nelson & Sauer, 1984; Klepinger *et al.*, 1986; Kyle, 1986; Piepenbrink, 1986; Pate & Hutton, 1988; Schoeninger *et al.*, 1989; Sillen, 1989; Pate *et al.*, 1991; Price *et al.*, 1992);
- intra- and inter-skeletal variation (Brätter *et al.*, 1977; Grupe, 1988);
- comparison of archaeological and modern bone (Hancock *et al.*, 1987, 1989);

- controlled feeding experiments with animals (Klepinger, 1990; Lambert & Weydert-Homeyer, 1993a, b).

In a sense, the field of bone chemistry developed in an almost reverse direction, with applications of the as yet unproven techniques initially preceding more fundamental studies of the underlying assumptions.

In addition to the post-mortem processes that obscure the original biogenic signal, various *ante-mortem* factors are known to affect the trace element composition of bone, which makes interpretation of bone chemistry data considerably more complex (Aufderheide, 1989; Sandford, 1993). Among these factors are an individual's age, sex and health. Furthermore, in addition to nutritional status, physiological status, e.g., pregnancy and lactation, plays an important role (Price *et al.*, 1986; Blakely, 1989). Trace element metabolism, synergistic and antagonistic interactions between different trace elements, or between elements and other food components (e.g., phytate, fibres) at the level of absorption in the gut, all contribute to the complexity (Mertz, 1987; Groff *et al.*, 1995). Many of the more dynamic aspects of bone chemistry during life are still incompletely understood. However, it has become clear that these relationships are crucial to our understanding of diet based on bone elemental composition (Sandford, 1993).

Modern trace element studies (e.g., Schutkowski, 1995; Schutkowski & Herrmann, 1996; Baraybar & de la Rua, 1997; Safont *et al.*, 1998; Baraybar, 1999; Schutkowski *et al.*, 1999) employ more elaborate frameworks for interpretation of the bone chemistry data. The simple 'trace element-dietary component' relationships (e.g., that Cu and Zn are indicators of meat consumption) are often supplemented by complex dietary schemes, with attention to other food components that are known to interact with trace element uptake, such as fibres and phytate or the calcium content of certain foods. In addition to Sr and Ba, the only valid palaeodiet indicators according to Ezzo (1994a, b), several other elements are often used as indicators of diagenesis. Recently, however, it has been shown that some long-held assumptions about Sr and Ba are also in need of revision. Foods high in those elements were found to be disproportionately sensitive to the Ca component of the diet (Burton & Wright, 1995). Additionally, it appears that bone

Sr/Ca ratios are not able to differentiate between the terrestrial and marine components of diet, contrary to earlier assumptions (Burton & Price, 1999).

During the last two decades, our awareness of the complexity of trace element biology and geochemistry, and the problems related to diagenetic alteration of buried remains, has increased substantially. However, much remains to be learned and many researchers are sceptical about the application of bone trace element analysis.

In contrast, stable isotope analysis has been more generally accepted as a valid technique by the anthropological/archaeological community. This can be partly explained by the fact that the theoretical models for stable isotope pathways in biological systems are better developed than for trace elements. Although the problem of diagenesis is of less concern for stable isotope analysis, several problems have also surfaced in this field. Because this thesis focuses on trace element analysis, these problems will not be discussed further here. For a discussion of these issues see for example Sillen *et al.*, (1989) and reviews by Schwarcz & Schoeninger (1991), Schoeninger & Moore (1992), Ambrose (1993), Pate (1994), Katzenberg & Harrison (1997) and Katzenberg (2000).

Recent years have seen considerable advances in the subfield of stable isotope analysis. Taking advantage of the progress made in other disciplines regarding isotope pathways, and the development of increasingly sophisticated sample preparation methods and analytical instrumentation (discussed in Katzenberg, 2000), researchers are now able to ask very different questions. Stable isotope analysis now often includes not only isotopes of C and N, but also of Sr, lead (Pb), and oxygen (O). The range of applications has been expanded beyond palaeodiet, to include reconstructions of climate (Fricke *et al.*, 1995), patterns of prehistoric mobility, including identification of immigrants (Ericson, 1985; Verano & DeNiro, 1993; Price *et al.*, 1994, 2000; Grupe, 1995; Ezzo *et al.*, 1997; Grupe *et al.*, 1997; Richards *et al.*, 1998; White *et al.*, 1998) and weaning patterns (Katzenberg *et al.*, 1996; Schurr, 1997; Wright & Schwarcz, 1998, 1999). In general, there has been an increase in the use of teeth for stable isotope analysis because of their higher resistance to diagenetic processes (Bell *et al.*, 1991; but for some recent studies of diagenesis in enamel see Kohn *et al.*, 1999; Sponheimer & Lee-Thorp, 1999) and the development of techniques for extraction of isotopes from apatite (see e.g., Lee-Thorp & Van der Merwe, 1987; Lee-Thorp *et al.*, 1989). Interestingly, some of these stable isotope techniques are applied to samples of teeth *and* bone, making use of the fact that tooth and bone samples of the same individual will provide information about diet and environment during infancy/childhood and adulthood, respectively (Sealy *et al.*, 1995).

Trace elements in dental enamel

The susceptibility of bone to diagenesis, and remodelling processes during life, play a much smaller role in the case of dental enamel. Nevertheless, anthropological studies of dental trace element composition have been relatively few in number, compared to the massive literature on bone chemistry (e.g., Hoyme & Koritzer, 1976; Koritzer, 1976; Boaz & Hampel, 1978; Elias, 1980; Schneider, 1984, 1986, 1988; Molleson, 1988; Schneider & Blakeslee, 1990; Gleń-Haduch *et al.*, 1997). To some extent this can be attributed to the scepticism generated by the problems encountered with bone trace element analysis. In addition, Sillen & Kavanagh (1982) have argued that, since less is known about the formation and maturation of teeth, the use of enamel samples for dietary reconstructions is even more complex than that of bone.

The clinical literature, however, offers a wealth of information about trace elements in dental tissues. Numerous studies in this area have been carried out over decades by dentists and dental biochemists because of the clinical relevance of dental trace elements in relation to caries. Although dietary reconstructions are not among the objectives, dietary intake is often a variable used in the explanation of differences in elemental levels between populations. Additionally, the results from several studies hint at other potential applications of trace element analysis that are definitely of interest to physical anthropologists. Examples include the relationship between trace elements and dental morphology and dental health. In the following, a brief overview of clinical studies of trace elements in enamel will be given. This overview is not meant to be exhaustive, but serves to illustrate the scope and variety of research carried out in this area.

Dental trace element studies in the clinical literature

Studies of the chemical composition of teeth initially focused on the major constituents of hydroxyapatite, such as Ca, phosphorus (P), and carbonate (e.g., Naujoks *et al.*, 1967), and on a limited number of minor elements (Sodium (Na), magnesium (Mg), chlorine (Cl)) which could be easily determined (Curzon & Featherstone, 1983). In addition, density studies of the different dental tissues, as well as of different regions within the tissues, for both permanent and deciduous teeth, were carried out. Most of these studies were aimed at understanding why certain areas are more likely than others to develop carious lesions.

With the development of new analytical techniques, a large number of the so-called 'trace' components in enamel could be studied. Trace elements are usually defined as those elements occurring in concentrations of less than 100 ppm (0.01%) in the body (Curzon, 1983). It should be noted, however, that some of the elements in this category may in fact occur in much higher concentrations in the hard tissues. For example, the hard tissues contain 70, 90 and 99% of the total body amounts of Mg, Pb, and Sr, respectively (Curzon & Cutress, 1983). Losee *et al.* (1974b) determined that at least 41 elements from the periodic table regularly occur in enamel. Although most of these elements have no known function in enamel, they may have an effect on the structural and chemical characteristics of the tissue (Curzon & Featherstone, 1983), and as such increase or decrease the susceptibility to caries (e.g., LeGeros *et al.*, 1977; LeGeros & Tung, 1983). The cariostatic effects of fluoride are well known. Several major epidemiological studies were carried out with respect to caries frequencies and their relationship to trace element levels in the environment, and animal experiments were set up to serve as model systems. These and many other studies are reviewed by Curzon & Cutress in their book *Trace Elements and Dental Disease* (1983).

Since caries is mainly a local surface phenomenon, it was necessary to gain some insights into compositional differences between different regions within enamel. Different microanalytical techniques have been used to study small areas of dental tissues, such as: secondary ion mass spectrometry (SIMS[2]), also referred to as ion probe microanalysis (Frostell *et al.*, 1977); microsampling by abrasion (Weatherell *et al.*, 1985; Murakami *et al.*, 1987); electron probe micro analysis (EPMA - Hals & Selvig, 1977; Selvig & Hals, 1977; Tötdal & Hals, 1985). These studies showed that for several elements a concentration gradient exists, with some elements showing higher concentrations near the surface, gradually decreasing towards the dentine (e.g., F, Cl, Pb, Zn, iron (Fe), antimony (Sb)), and others showing an opposite trend (e.g., Na, Mg, CO_3). Other elements (e.g., Sr, Cu, aluminum (Al), potassium (K)) show a more uniform distribution throughout the enamel (Frostell *et al.*, 1977; Jenkins, 1978; Ishiguro *et al.*, 1994). Such patterns have also been found for dentine and cementum (Frostell *et al.*, 1977; Murakami *et al.*, 1987; Nakagaki *et al.*, 1988; Kato *et al.*, 1992; Ishiguro *et al.*, 1994). The observed concentration profiles have been explained as being the result of diffusion. The outer layers of enamel accumulate elements from the environment that bind to apatite, whereas non-binding elements will not be retained. The concentrations of the strongly binding elements in the outer layers will tend to be higher with increasing duration of exposure to the element-containing fluids in the oral cavity (Jenkins, 1978).

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[2] A list of abbreviations used in this thesis is included in the prefatory pages.

Some interesting studies have been carried out, mostly during the 60's and 70's, in which the effects of trace elements on dental morphology and structure were investigated. For example, Kruger (1962, 1966) carried out experiments with rats to study the effects on molar morphology of boron (B), F and molybdenum (Mo), injected during different periods of dental development. The effects of the elements depended upon the time of administration relative to development of the molars. Injections of B and F yielded teeth with wider and shallower mesial fissures, and, with B, the fissures were in some cases completely eliminated. Fluoride also produced thinner layers of dentine and enamel in maxillary molars, and reduced the maximum mesio-distal diameter of these teeth. Molybdenum by itself was reported as having no significant effects on morphology. Similar results were found by Cooper & Ludwig (1965) in a study of the effect of fluoride on human molar morphology. A change in fissure patterns is considered one of the possible mechanisms through which F exerts a cariostatic effect. Shallower and wider fissures would reduce the chances of food retention and, thus, the risk of caries.

The effects of injections of Sr on rat molar morphology were studied by Castillo-Mercado & Bibby (1973) and Curzon *et al.* (1982). The first study reported a widening of the fissures, and both studies found a thickening of the dentine.

Some feeding experiments with animals are particularly interesting from a palaeo-nutrition point of view. Healy & Ludwig (1968), for example, raised twin sheep on pastures that differed with regard to the barium content of the soils. They found that the difference in soil Ba content was carried through into differences in Ba content of various tissues, including dentine and enamel. Molybdenum has also been found to accumulate in teeth when dietary levels of this element increase (Mills & Davis, 1987). Wolf *et al.* (1973) analyzed the bones and teeth of human fetuses from different regions for their Sr content. The Sr concentration in these tissues showed a correlation with the Sr content of the drinking water in the different regions. This study demonstrated that the Sr levels in fetal tissues are directly related to the amount of this element ingested by the mother. These results were in agreement with those from other studies with mice (cited in Wolf *et al.*, 1973), in which it was demonstrated that Sr could reach the young through the placenta.

Because teeth contain a permanent record of the circumstances during dental development, they form excellent samples to monitor environmental exposure to toxic substances in modern populations (Sharon & Ryge, 1984; Sharon, 1988). In order to determine 'base levels' of heavy metals such as Pb and cadmium (Cd), archaeological samples are often studied for comparison with modern samples.

Grandjean *et al.* (1979) analyzed archaeological bone and tooth samples from Sudanese Nubia (3300 B.C.- 750 A.D.) and similar samples from present-day Denmark for their Pb concentrations. The comparison showed that modern Pb values for bone were about ten times higher than those measured for the oldest Nubian samples. The tooth samples showed a 30-fold increase between the oldest Nubian and present-day Danish samples. A slight increase in Pb levels for Nubian samples over time appears to be correlated with the introduction of lead technology to the area. In a similar study, Ericson *et al.* (1979) analyzed skeletal concentrations of Pb in ancient Peruvians. The results showed that modern populations in the industrialized countries are exposed to such significant contamination that skeletal Pb levels are elevated to about 500 times the natural levels. Clearly, current standards for Pb exposure, referred to as 'normal', are much higher than the natural environmental load to which humans were exposed prior to the industrial use of Pb (Grandjean *et al.*, 1979).

Kuhnlein & Calloway (1977) studied the concentrations of Pb, Sr, Zn, Cu and mercury (Hg) in pre-industrial and contemporary Hopi Indians in Northeastern Arizona. Their samples consisted of dentine, removed from the inside of the crowns of deciduous teeth. Because dentine is continuously deposited throughout life, the concentrations of the elements in these samples were interpreted as being representative of the number of years of exposure, this being equal to the number of years the tooth was in use before being shed. They compared the Hopi samples with similar samples from modern suburban California. Lead, Zn and Cu were found to be higher in the modern teeth, corresponding to higher environmental levels of these elements. Mercury occurred in similar concentrations in all groups. The results for Sr were interesting since they were found to be more than four times higher in the 17th century groups. This could be explained by the presence of high natural levels of Sr in Northern Arizona, and the traditional Hopi practice of adding the ash of green plants during the preparation of cornmeal products.

These and similar studies (e.g., Attramadal & Jonsen, 1978; Whittaker & Stack, 1984; Eide *et al.*, 1993) indicate the importance of teeth for studies of environmental exposure to pollutants. In addition, Kuhnlein & Calloway's study illustrates how trace element analysis of teeth can help us to understand not only environmental factors but also aspects of diet of past populations.

Dental trace element studies in the anthropological literature

In one of the first anthropological studies of dental trace elements, Koritzer (1976; Hoyme & Koritzer, 1976) reported on an exploratory analysis of dental enamel to determine the potential of such studies for archaeology. He determined the concentration of 12 trace elements in the enamel of 43 prehistoric Amerindians from Illinois, Maryland and Virginia and reported inter- and intra-group differences in enamel composition by geographic location, sex and age groups. Previously reported data (Hoyme (1972) and Koritzer (1972), cited in Hoyme & Koritzer, 1976) had shown clear differences in dental pathology for these same populations by age, sex, and geographic locality. However, to my knowledge, a comparison of data on dental pathology with results of chemical analyses has never been published.

Schneider (1984, 1986) attempted to explain differences in caries frequencies between six populations of prehistoric Amerindians from Ohio with a different subsistence base (hunting-gathering, horticulture, and mixed subsistence) in terms of the trace element composition of enamel. Using correspondence analysis, she was able to discriminate between samples on the basis of subsistence system (although the horticultural samples themselves showed considerable variation), and geographic locality. This division of groups turned out to correspond with differential caries experience. On the basis of these data she concluded that Zn, Cu and Fe have a cariostatic effect and that other elements, especially nickel (Ni), have a cariogenic effect.

Schneider also analyzed isolated permanent and deciduous teeth from the Grimsby Cemetery (Ontario, Canada) in order to establish whether there was any indication for dietary stress (Schneider, 1988). She was able to separate both the permanent and deciduous samples by tooth type using correspondence analysis. She hypothesized that these differences in composition may be due to differences in developmental period for each tooth type, and thus be related to dietary intake. In addition, she concluded that the compositional profile of the samples was comparable to other maize-dependent horticultural groups. In association with this maize-focused diet, the Grimsby population may have experienced Zn and Fe deficiencies due to interference of the uptake of these elements by the phytic acid in corn. Copper was found to be absent in the deciduous teeth, and Schneider suggested that this might have resulted in anaemia due to the role of Cu in the oxidation of ferrous to ferric Fe (see also Groff *et al.*, 1995).

Schneider & Blakeslee (1990) used trace element composition of enamel to study residential mobility patterns in four prehistoric Arikara populations. The trace elements used in their study were chosen based on their demonstrated capacity to discriminate between populations according to subsistence system and geographic region (Schneider, 1984, 1986). Within each population, individuals appeared to cluster especially by age and sex. The authors suggested that the observed differences between the male, female and subadult groups for the different sites represent a specific pattern of residential mobility involving out-migration for males.

The results of the studies above suggest that it is possible to find relationships between dental trace element composition and subsistence, and that individuals can be clustered on the basis of dental composition both within and between groups. However, the results from several studies of dental Sr concentrations for dietary reconstructions have been less conclusive. Boaz & Hampel (1978) applied Sr-analysis to enamel samples of fossil hominids, in order to determine the relative contribution of meat and vegetable foods to their diet. The hominid samples were compared with enamel samples from modern and fossil herbivores and carnivores. Because 99% of total body Sr is concentrated in the hard tissues, carnivores eating the meat of their prey ingest relatively low amounts of Sr. Thus, the modern hyaena, *Crocuta crocuta*, was expected to show the lowest Sr content based on its carnivore status, but in fact showed the highest of the modern mammals that were studied. These results led the authors to conclude that Sr was probably susceptible to diagenetic alteration, and therefore not suitable to reconstruct the diet of fossil taxa. However, hyaenas are renowned bone-crushers, and have been seen to swallow entire bones or bone parts (Sutcliffe, 1970; Marean, 1995). This could in fact mean that hyaenas, contrary to other carnivores, ingest quite a substantial amount of Sr, which could explain the high Sr concentrations in hyaena enamel. This would in turn suggest that the Sr content of enamel does indeed reflect dietary intake.

Elias (1980) discussed Boaz & Hampel's study and pointed out some problems with the study of Sr as a palaeodietary indicator. He drew attention to the possibility of species-specific drinking habits that could cause differential ingestion of Sr, and also potential physiological differences that might affect Sr metabolism. Therefore, for his own study of Sr in enamel samples from vegetarian and non-vegetarian human groups in India, he chose his samples from a single geographic locality so that differences in Sr intake via drinking water could be excluded. However, he was not able to distinguish between the two groups on the basis of the Sr content of enamel. He argued that the very similar values of Sr were most likely due to the fact that vegetables, consumed by both groups, are such predominant sources for Sr that meat consumption cannot be detected. He concluded that the *degree* of vegetable intake could not accurately be monitored in fossil humans using Sr as an indicator. However, it has been shown that the bulk of Sr intake in human diet comes from drinking water rather than foods (Wolf *et al.*, 1973; studies reviewed in Cutress, 1983). Therefore, a similarity in Sr-levels between the two populations might have been expected irrespective of their food intake.

The research potential of dental trace elements

The above discussion offers a glimpse of various exciting possibilities for the application of dental trace element analysis in anthropology. The results of the clinical studies suggest that a number of trace elements in enamel reflect dietary intake (e.g., Ba, Sr, Mo). In addition, some trace elements have been found to affect morphological characteristics of the tooth crowns (e.g., F, Mo, Sr), and susceptibility to caries. Possible effects of trace elements on fissure patterns, dentine/enamel thickness, and frequency of caries could provide interesting new avenues in the study of dental palaeopathology. Several other studies have shown that tooth composition can offer clues about exposure to environmental contaminants and, possibly, patterns of mobility. The research potential is further augmented because of the developmental pattern of teeth and their unique incremental structure. As was mentioned before, these characteristics may allow us to narrow down dietary changes or other life history variables to relatively specific age ranges. The key to unlocking this potential, however, is our understanding of the relationship between trace element intake and dental trace element composition.

The historical development of bone chemistry studies has shown that fundamental issues need to be addressed and understood prior to successful applications of the techniques to archaeological samples. The work done by Boaz & Hampel (1978) and Elias (1980), discussed above, emphasises the need for a better understanding of trace element metabolism, physiology and environmental routing - e.g., does a certain trace element enter the body mainly via food or water, or other pathways? Many of the complications that arose in the field of bone trace element studies apply to the analysis of dental trace elements as well. Although diagenesis is of less concern in the case of tooth samples, proper care must be taken when analyzing archaeological tooth samples. Whereas normally the trace elements in enamel are incorporated during formation and during post-eruptive maturation only, in buried teeth ions can still be exchanged between the outer layers of enamel and the soil. A careful analysis of the elemental distribution patterns is necessary in order to identify the contribution of each of these three different time periods (Molleson, 1988). Many studies have focused on determining the variation in levels of trace elements in enamel from different individuals or populations. However, if we want to use trace element concentrations in enamel as a tool in palaeodietary studies, we will need to ask more precise questions: *how* and *when* are the trace elements built into enamel, and *to what extent* do trace element levels in enamel reflect the trace element availability in the diet?

Sillen & Kavanagh (1982) more or less discouraged the study of tooth composition arguing that our understanding of the chemical aspects of tooth formation is limited. Furthermore, teeth are generally extremely valuable in many other types of anthropological analyses, and their destruction for chemical analysis cannot be justified unless a clear framework for the interpretation of the results is established. However, since Sillen & Kavanagh's publication there have been numerous advances in the areas of dental biochemistry and dental development. Both anthropologists and dentists have carried out more detailed studies of the incremental growth of enamel and dentine, and more information is available about the formation times of tooth crowns (see Chapter 2). Furthermore, the development of more sensitive (micro)analytical techniques, such as laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), makes it possible to take relatively small samples. This, combined with the increase in interest in the use of archaeological tooth samples for various research questions, suggests the time is appropriate for an inventory of what we *do* know at this moment.

It is important to remember that the composition of teeth will reflect aspects of the diet of infants and children rather than adults. Many of the studies carried out so far have focused on questions that required the analysis of adult bones, such as differential dietary intake based on sex or status, or general subsistence patterns. Infant and child nutrition has in general received much less attention. However, a better understanding of the health and nutrition of this part of the population could provide important insights into population dynamics and adaptation. This is illustrated by the increasing number of studies concerned with the reconstruction of weaning practices in prehistoric populations (see e.g., Sillen & Smith, 1984; Katzenberg *et al.*, 1996; Schurr, 1997, 1998; Herring *et al.*, 1998; Wright & Schwarcz, 1998, 1999).

Why study infant nutrition and weaning?

From birth until at least 6 months of age, breast milk is generally the only food an infant will receive. Human milk is considered a complete food, containing all the necessary nutrients for proper growth and health for infants. In addition to the proteins, lipids, carbohydrates and minerals an infant receives through breast milk, various important immunological factors are transferred between the mother and infant via milk (Subcommittee on Nutrition during Lactation, 1991; Newman, 1995). The infant's immune system is still developing during the first months of life. Therefore, the passive immunity obtained via breast milk plays a crucial role in the infant's ability to resist pathogens to which it is exposed in its environment. Weaning of infants prior to the full development of their own immune system could thus seriously compromise their chances of survival. In addition, breastfeeding suppresses the return of ovulation in the mother, thereby affecting fertility. Because of these effects on fertility and infant morbidity and mortality, age at weaning is an important palaeodemographic variable (Katzenberg *et al.*, 1996; Herring *et al.*, 1998).

The general pattern for many pre-industrial populations of agriculturalists is that mortality is relatively high during infancy, and then declines during childhood (Saunders, 1992). Post-neonatal mortality is to a large extent due to environmental factors, including poor sanitation and inadequate nutrition (Saunders, 1992). In addition to the obvious detrimental effects of poor nutrition on the health of the infant, the process of weaning has often been considered a considerable physiological stress factor (Katzenberg *et al.*, 1996). In fact, before biochemical methods for determination of weaning age were developed, non-specific osteological indicators of stress, such as disturbances in enamel formation (e.g., linear enamel hypoplasia or LEH) were used for this purpose. The relationship between weaning and dental hypoplasia is, however, sometimes ambiguous (Blakey *et al.*, 1994) and the biochemical methods provide a more direct method of determining weaning age.

Recent studies of weaning patterns are based on stable isotopes of C, O and N from either bone or teeth (e.g., Katzenberg *et al.*, 1996; Schurr, 1997; Wright & Schwarcz, 1998, 1999), but Sr/Ca ratios have also been used (Sillen & Smith; 1984).

These methods are based on the fact that breast milk and solid foods contain different isotopic and trace element signatures. Whereas stable isotopes can provide us with information about general patterns in dietary intake, trace elements could theoretically provide more specific information, for example about deficiencies in certain nutrients. A number of trace elements (e.g., Zn, Cu, Fe) play crucial roles in biological processes, and deficiencies of these elements can in some cases lead to skeletal anomalies or disturbances in biochemical reactions (e.g., Mertz, 1987) which may cause disease or sometimes even death.

Finally, using teeth rather than bone is particularly advantageous for the study of infants since infant bones present additional complications. Immature bones are less dense than their mature counterparts and the cortex is much thinner. Because of this, infant bones are even more susceptible to diagenesis than adult bones. In addition, infant bones show extensive remodelling after birth (Gordon & Buikstra, 1981; Sillen & Kavanagh, 1982; Lambert *et al.*, 1985; Vuorinen *et al.*, 1990). Both deciduous and permanent teeth will preserve the original biogenic signal to a much greater extent than bone. However, there is an important difference between the two types of dentition. Deciduous teeth will be available from infants and young children who perished at a young age. In contrast, the permanent teeth will represent the individuals who survived past childhood (Grupe, 1998). A comparison of these two groups within one population might give us important insights into aspects of health and nutrition during the crucial early stages of life.

Research objectives

This study has two objectives: To develop a general model for trace element incorporation in teeth, and to analyze tooth samples from individuals of known dietary history. Subsequently, the results of the analyses will be compared with the general model. These two objectives are discussed in more detail below:

1. Theoretical aspects

Investigation of factors that determine dental trace element composition, and development of a model which will serve as an interpretive framework for trace element data from tooth samples.

A major part of the thesis is concerned with the development of a general model that will describe the pathways of trace elements into deciduous and permanent tooth enamel. Information available in the nutritional and medical literature will be used in conjunction with our current understanding of dental development and enamel maturation processes.

For the deciduous dentition, the first crowns (incisors) begin to develop around 3-4 months gestation. The last deciduous teeth to form, the second molars, complete the process of crown formation around the end of the first year of life. Although most of the development of the permanent teeth takes place postnatally until around 12-16 years of age (for M3), development of the permanent first molar is usually initiated prior to birth (Avery, 1992; Hillson, 1996).

During gestation, the developing fetus (and thus, all its tissues including the forming teeth) depends entirely on the mother for its nutrient supply via the placenta. The composition of the parts of the deciduous teeth and permanent first molar that are formed *in utero* is therefore determined by the composition of maternal blood. In addition, filtering effects take place during the transfer of nutrients between mother and fetus. For example, Sr is discriminated against in favour of Ca during transport across the placenta (Comar *et al.*, 1957; Comar & Wasserman, 1964).

Generally, after birth the infant depends on the mother for its nutrition via breast milk for at least some time. During this period the infant's nutrition is determined by the composition of the milk, which in turn depends, amongst other factors, on maternal blood composition. As is the case for placental transport, filtering effects take place during the transfer of nutrients from mother to infant across the mammary gland.

Once complementary foods are introduced and the infant is gradually weaned, other foods and liquids, as well as environmental exposure to contaminants, begin to play a role in the infant's dietary (and thus trace element) intake. As long as the teeth are still developing and protected in their bony crypts, trace elements will reach the enamel primarily via the blood supply. However, once the teeth have erupted, exposure to foods, liquids and contaminants also takes place directly in the oral cavity. This applies to both deciduous and permanent teeth, and will mostly affect the outermost layers of enamel.

Figure 1.1 illustrates in a simplified form the relations among maternal, fetal and infant nutritional status and the 'barriers' represented by placenta and mammary gland, and provides a diagrammatic representation of the basic components of the model that will be developed in this thesis.

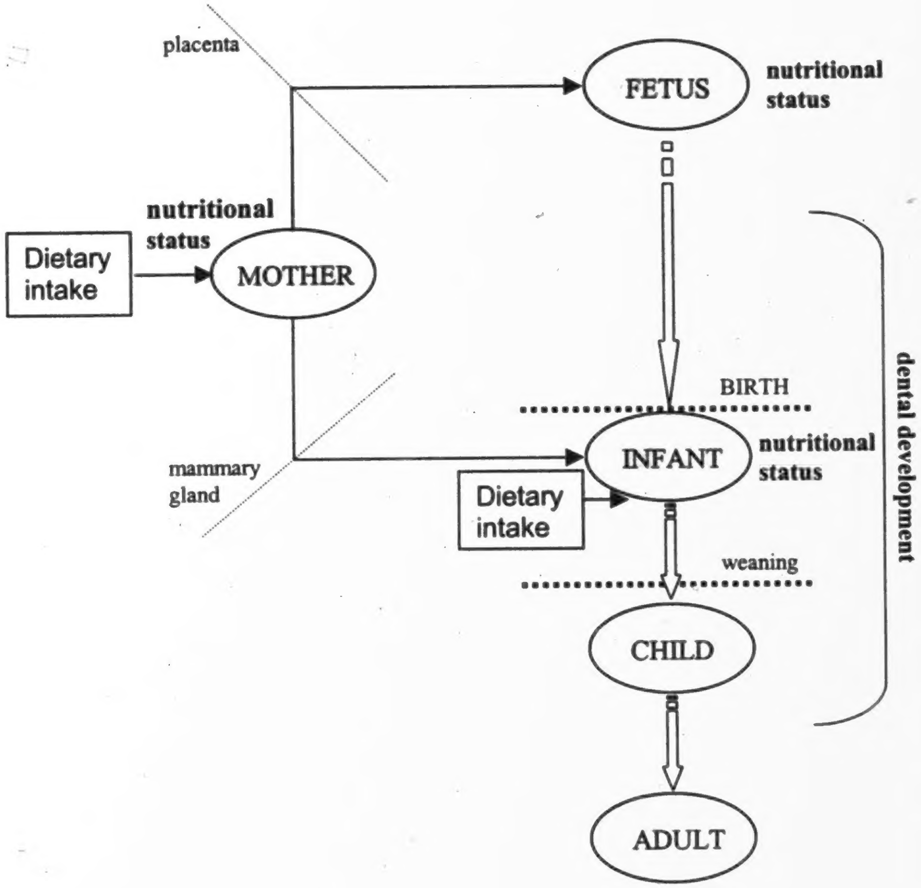
placenta
FETUS
nutritional status
nutritional status
Dietary intake
MOTHER
BIRTH
mammary gland
INFANT
nutritional status
Dietary intake
weaning
CHILD
ADULT
dental development

Fig. 1.1: Diagrammatic representation of the basic components of the model which will be developed in this thesis. Shown are the simplified relations between maternal, fetal and infant nutritional status, and the barriers formed by the placenta and mammary gland. Dental development takes place in utero, and during infancy and early childhood.

Obviously, much of what has been written about trace element metabolism and physiology in the context of bone trace element analysis also applies to the use of trace elements from dental tissues (Grupe, 1998). However, the fundamental differences between bone and teeth require us to evaluate the existing information from a different perspective, and to add information specific for teeth.

Chapter 2 will examine dental development from a trace element perspective and a similar approach is used in Chapter 3, where aspects of infant nutrition are discussed. The information provided in these chapters will be used to construct a more detailed model to describe the relations between trace element uptake *in utero* and during infancy, and trace element composition of enamel.

2. Practical aspects

The analysis of deciduous tooth samples from individuals of known dietary history to test several aspects of the model, and analysis of permanent teeth from an archaeological specimen.

Although some of the anthropological studies of dental trace elements were able to demonstrate relationships between subsistence and dental composition (see above), these were based on archaeological material. Such samples are not very suitable for the purpose of testing some assumptions involved in the model because relevant information regarding diet is lacking. For obvious reasons we cannot carry out 'controlled feeding experiments' with human subjects to unravel the fundamental relationship between diet and tooth composition. However, the analysis of samples of known dietary intake can help us to determine if, and how, dietary intake might be reconstructed from dental trace element composition.

For this study, a unique sample of modern deciduous teeth was available, consisting of three almost complete dentitions from individuals for whom some records regarding dietary intake, age of weaning, health, eruption and shedding of individual teeth were available. In addition, the teeth from two other individuals, including some information about age of weaning and weaning foods were available for study. These samples are the closest possible alternative to controlled samples, as the available records can in fact provide us with some degree of feedback to assist in the interpretation of obtained compositional data. .

Several trace elements were selected from the palaeodietary literature as potential indicators of various food types. The permanent teeth from an archaeological specimen were analyzed with a bulk technique (neutron activation analysis – NAA) and a microanalytical technique (laser ablation inductively coupled plasma mass spectrometry – LA-ICP-MS). The deciduous tooth samples were analyzed with LA-ICP-MS only. Continuous laser scans were performed across the tooth, both in a cross-sectional direction (inner dentine to outer enamel) and longitudinal direction (top of crown towards cementum-enamel junction). The longitudinal lines follow the temporal axis of crown formation, resulting in trace element vs. time profiles. For one of the deciduous molars, two-dimensional elemental distribution maps were also collected. The elements selected for analysis are: Ca, P, Ba, Sr, Zn, Cu, Fe, Mn, Mo, vanadium (V), and Pb.

Chapter 4 covers the materials and methods used, and the results are presented and discussed in Chapter 5, using the model presented in Chapter 3 as a reference. A conclusion and suggestions for future studies are given in Chapter 6.

CHAPTER 2

DEVELOPMENT, STRUCTURE AND COMPOSITION OF ENAMEL

Introduction

The formation of teeth, and especially the development of enamel – the hardest biological tissue known (Aiello & Dean, 1990) – has fascinated researchers for a long time. Nevertheless, even with the advanced techniques available to us today, many questions about tooth formation remain. This is partly due to the difficulties of studying enamel during its formation; for example, in *demineralized* sections one can no longer observe the forming enamel, whereas in *undemineralized* sections the cells forming the enamel are lost, so that the cellular processes are no longer visible (Ten Cate, 1994). These and other problems in the areas of dental research necessarily impose restrictions on the model developed in this thesis. Both the timing of developmental processes, and aspects of enamel structure and composition are essential components of the model to be examined.

Below, I will present a brief overview of dental development and dental biochemistry as it is currently understood. The emphasis will be on aspects that are important in the context of this study, such as mineral uptake by the ameloblasts throughout the different stages of dental development. Although the majority of studies do not provide temporal data, an attempt is made to provide time frames for the various processes and stages, because this is of crucial importance for palaeodietary applications of enamel trace element composition.

Dental development – an overview

In humans, two sets of dentition develop: the primary or deciduous dentition (20 teeth), and the secondary or permanent dentition (32 teeth). There is no absolute separation in developmental time between the two dentitions (Fig. 2.1). In fact, some authors have argued that, at least from an ontogenetic perspective, the permanent molars are primary teeth, since, unlike the other permanent teeth but like deciduous teeth, they have no predecessors (Schwartz & Langdon, 1991).

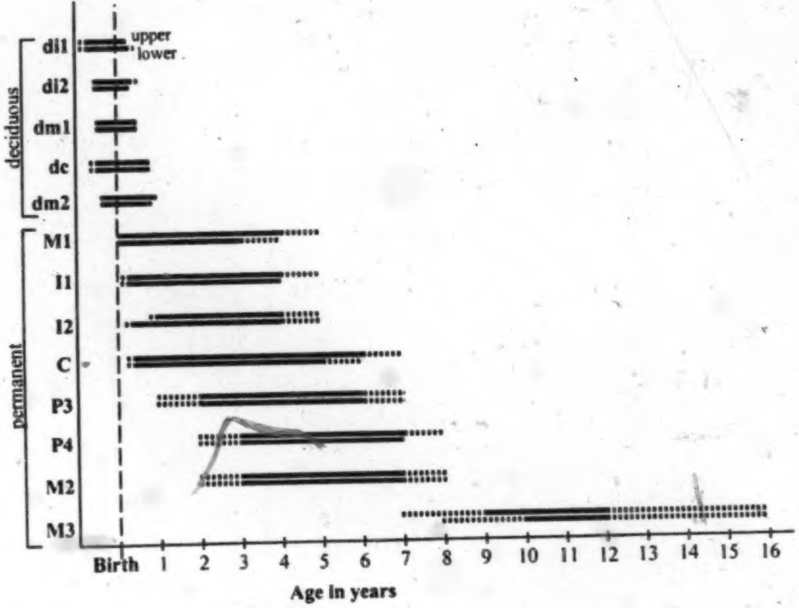

Fig. 2.1: Development of the deciduous and permanent dentition. Each tooth is represented by two bars, for the upper and lower teeth, which indicate the period of crown formation. Stippled lines indicate the range for initiation of calcification or completion of calcification.

Each individual tooth has a distinctive developmental period during which the crown is formed, a root is developed, and eruption into the oral cavity takes place. Although the various teeth form during different periods, in different locations along the jaw, and differentiate morphologically into different shapes, the processes of tooth development are fundamentally the same for all teeth of the deciduous and permanent dentition.

Initiation of development

Dental development is initiated as early as 6-8 weeks *in utero* with the formation of a thickened band of epithelium that lines the primitive oral cavity in the shape of the future dental arcades (Hillson, 1996). The epithelial cells proliferate and finally grow into the underlying ectomesenchym. These outgrowths give rise to the dental laminae, which eventually form the tooth germs (Fig. 2.2). These tooth germs go through a series of developmental stages, whereby the shape of the tooth germ gradually evolves from bud to cap to bell (Fig. 2.3). This division into separate stages is rather arbitrary, as they represent a continuum of development (Avery, 1992; Ten Cate, 1994).

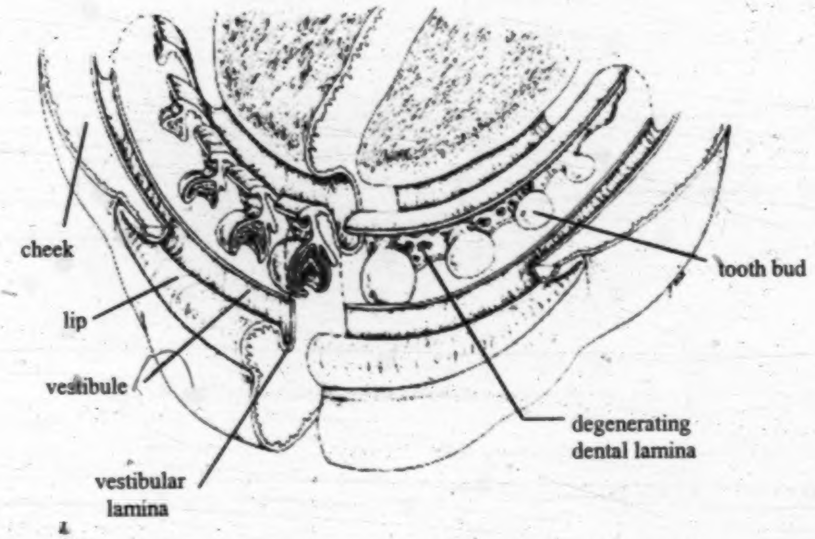

Fig. 2.2: The dental laminae and developing tooth buds for the mandible. (Reprinted with permission from Avery, 1992).

The bud stage is relatively short and represents a continued process of epithelial cell division, which leads to an increase in the volume of the tooth bud. During the bell stage, several different functional units can already be recognized (Fig. 2.3): 1) the cells of the enamel organ (sometimes called the dental organ) that will play a role in the formation of enamel, 2) the dental papilla that will form dentine and pulp, and 3) the dental follicle that will give rise to supporting connective tissues (Avery, 1992; Ten Cate, 1994).

As noted above, the dental lamina is formed at about 7 weeks *in utero*, and this means that the initiation of the development of the entire deciduous dentition takes place simultaneously. By 10 weeks, the enamel organs for all deciduous teeth have been formed (Hillson, 1996). The successional permanent teeth (*i.e.*, incisors, canines, and premolars) are initiated between the 20th week *in utero* and 10 months after birth, and the permanent molars between the 20th week *in utero* for M1 and the 5th year of life for M3 (Ten Cate, 1994).

The cells of the internal enamel epithelium, which differentiate into enamel-forming ameloblasts, have several different functions, which they perform in succession (Ten Cate, 1994):

a) formation of the outline of the crown

b) induction of differentiation of the odontoblasts (dentine-forming cells)

c) secretion of matrix

d) maturation of the enamel

e) protection of the enamel

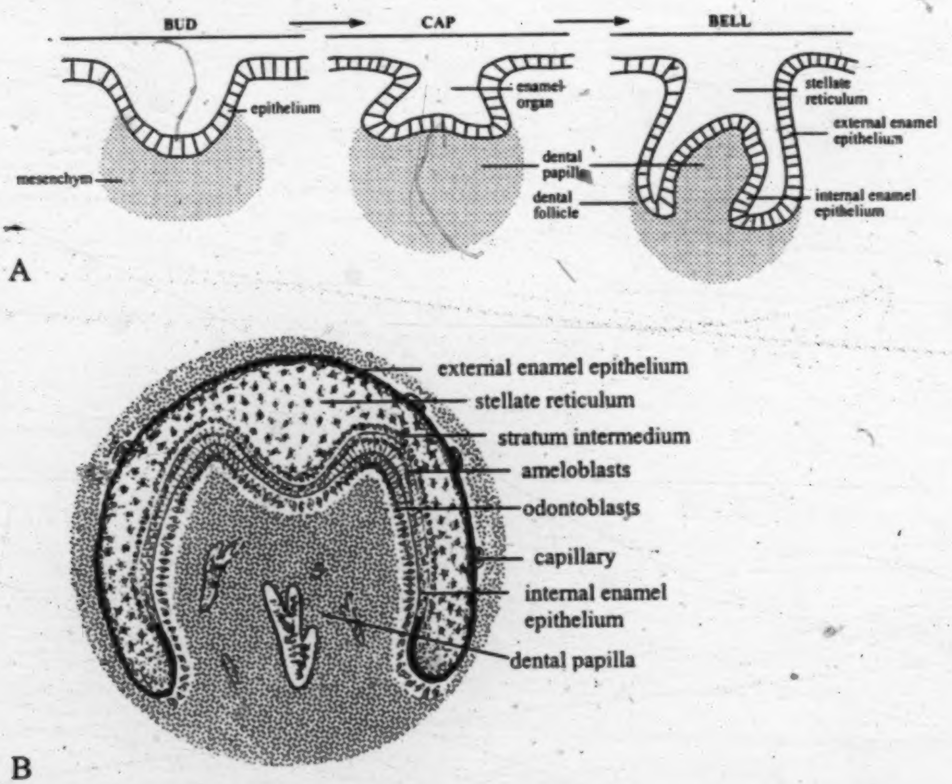
BUD
CAP
BELL
epithelium
mesenchym
enamel-organ
dental papilla
dental follicle
stellate reticulum
external enamel epithelium
internal enamel epithelium
A
external enamel epithelium
stellate reticulum
stratum intermedium
ameloblasts
odontoblasts
capillary
internal enamel epithelium
dental papilla
B

Fig. 2.3. A: Developmental stages of the tooth germ from bud to cap to bell (based on Avery, 1992). B: A more detailed diagram of the bell stage showing the different functional units (Reprinted with permission from Avery, 1992).

Crown formation

The outline of the crown (or, more accurately, of the future enamel-dentine junction (EDJ) - Fig. 2.4) is determined during the late bell stage, when the cells of the internal enamel epithelium at the points of the future cusps stop their process of cell division and enter the maturation stage. This will influence the cells in the underlying dental papilla, which then differentiate into odontoblasts and begin the process of dentine formation by secreting matrix. This, in turn, has an inductive effect upon the now mature cells of the internal enamel epithelium, which then differentiate into ameloblasts. As soon as the first dentine is produced, the ameloblasts also enter the secretory stage and produce matrix against the newly formed dentine, thus forming the enamel-dentine junction (EDJ).

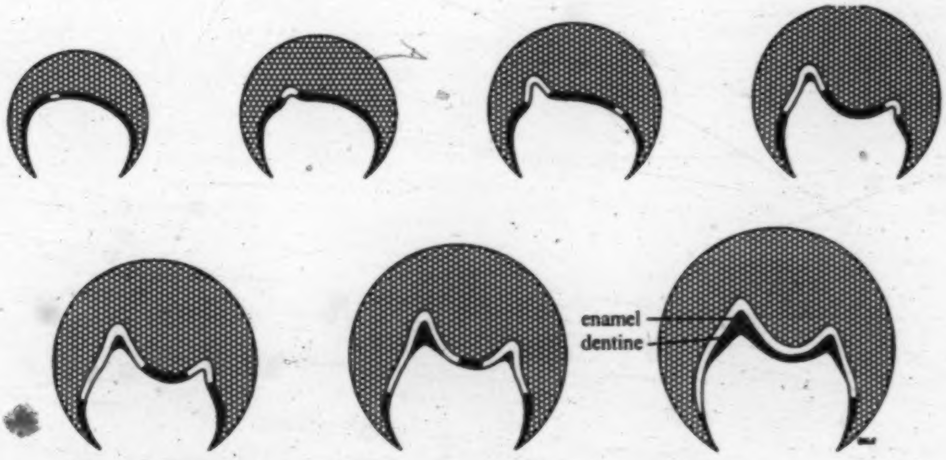

Fig. 2.4: Crown pattern formation in the internal enamel epithelium during the late bell stage. Dark areas: cell division; white areas: zone of maturation. Dentine formation proceeds inwards to the future pulp cavity, and is indicated by the shaded region. Enamel formation proceeds outward from the EDJ, indicated by the stippled area. (Reprinted with permission from Ten Cate, 1998).

The odontoblasts move away from the EDJ towards the future pulp cavity while leaving behind an increasing layer of dentine. At the same time, the ameloblasts move away from the EDJ in the opposite direction, while secreting enamel matrix (Fig. 2.5). The enamel matrix is almost immediately mineralized to about 30% by weight (wt%)(Jenkins, 1978; Ten Cate, 1994; Smith, 1998), apart from a more heavily mineralized layer adjacent to the dentine (Crabb & Darling, 1962; Suga, 1982, 1983, 1989).

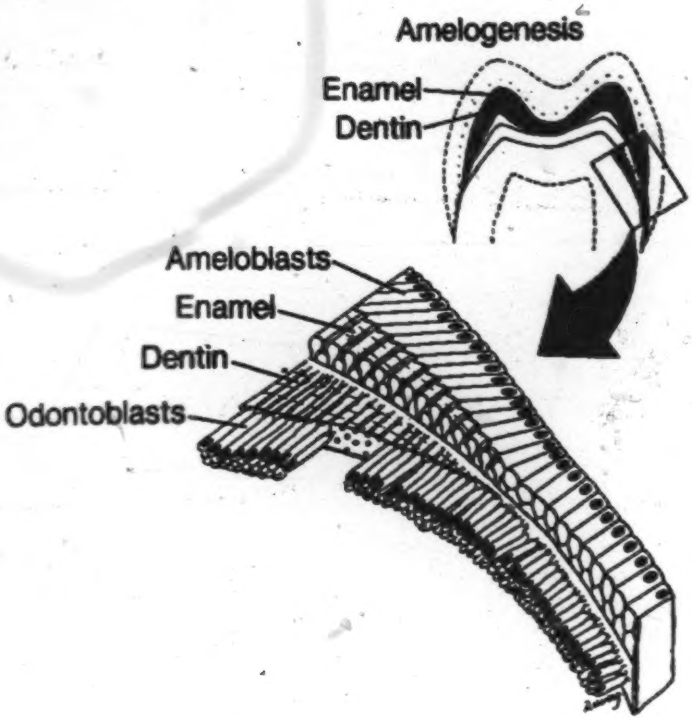

Fig. 2.5: During dental development, the ameloblasts and odontoblasts move away from the EDJ in opposite directions, while depositing enamel and dentine, respectively. (Reprinted with permission from Avery, 1992).

The timing of onset of tooth formation (*i.e.*, dentine/enamel matrix secretion) for the deciduous dentition is summarized in Table 2.1, showing the results from two different studies. These data show that initiation of crown formation for all tooth types takes place within a timeframe of only five weeks (15-20 weeks *in utero*). Because the enamel organs for all deciduous teeth are formed by about 10 weeks, it is clear that, for the deciduous dentition, it takes several weeks for each tooth bud to reach the stage of matrix deposition and initial mineralization.

Initiation of the first permanent molar crowns takes place in the last weeks before birth while the other permanent tooth types start enamel and dentine formation from 3-4 months after birth until 7-10 years for the M3. Tables 2.2 and 2.3 summarize the initiation and completion of crown formation for the deciduous and permanent dentition, respectively.

Table 2.1: Timing of onset of dentine/enamel formation for the deciduous teeth. For abbreviations di1, di2, etc. see List of Abbreviations included in the prefatory pages.

Tooth type	Kraus & Jordan (1965) (weeks *in utero*)	Sunderland *et al.* (1987) (weeks *in utero*)
di1	16	15
di2	18	16
dc	19	19
dm1	17	16
dm2	20	20

Table 2.2: Timing of crown initiation and completion for the deciduous dentition (based on table 6.2, Avery, 1992).

Crown formation times deciduous dentition		
Tooth type	**Initiation of calcification (mo *in utero*)**	**Completion of calcification (mo after birth)**
Lower di1	3-4	2-3
Upper di1	3-4	2
Lower di2	4	3
Upper di2	4	2-3
Lower dm1	4	6
Upper dm1	4	6
Lower dc	4-5	9
Upper dc	4-5	9
Lower dm2	5	10
Upper dm2	5	11

Table 2.3: Timing of crown initiation and completion for the permanent dentition (based on table 6.3, Avery, 1992). For abbreviations M1, I1, I2, etc. see List of Abbreviations.

Crown formation times permanent dentition		
Tooth type (eruption sequence)	**Initiation of calcification**	**Completion of calcification (yr)**
Lower M1	birth	3-4
Upper M1	birth	4-5
Lower I1	3-4 mo	4
Upper I1	3-4 mo	4-5
Lower I2	3-4 mo	4-5
Upper I2	10-12 mo	4-5
Lower C	4-5 mo	5-6
Upper C	4-5 mo	6-7
Lower P3	1-2 y	6-7
Upper P3	1-2 y	6-7
Lower P4	2-3 y	7
Upper P4	2-3 y	7-8
Lower M2	2-3 y	7-8
Upper M2	2-3 y	7-8
Lower M3	8-10 y	12-16
Upper M3	7-9 y	12-16

While matrix secretion takes place in the cuspal area of the crown, additional cells of the internal enamel epithelium mature and the area of matrix secretion gradually extends further in cervical direction, outlining the complete crown area (or actually: the EDJ). The rate of differentiation of ameloblasts further down the sides of the future crown is referred to as the *enamel extension rate* (Shellis, 1984). In deciduous teeth this rate may be relatively constant over the crown surface, while being about five times greater than in permanent teeth. In the permanent teeth the extension rate slows down towards the cervical area, so that the last part of the crown to be formed may take quite some time. This is important, because it means that equal proportions of the crown height do not represent similar amounts of developmental time (Shellis, 1984, 1998; Beynon *et al.*, 1998; Reid *et al.*, 1998; Liversidge, 2000).

Mineral transport during the initial stages of dental development

During the bud-cap-bell stages of enamel development, the cells of the internal enamel epithelium derive their nutrients from two different sources: the blood vessels in the dental papilla, and the vessels that are found along the external enamel epithelium. Once the dentine deposition has started, the blood vessels in the dental papilla can no longer supply the enamel-forming cells with nutrients. However, a subsequent collapse of the *stellate reticulum* (see Fig. 2.3b), which separates the internal from the external enamel epithelium, brings the internal enamel epithelium cells closer to the external enamel epithelium. For the remainder of enamel formation, the vessels along the external enamel epithelium supply the ameloblasts with nutrients (Ten Cate, 1994).

Many aspects of the transport of minerals from blood to ameloblasts, and then on to the forming enamel, are not understood at this point. The basics of the transport mechanisms for Ca and P, the major constituents, are still the subject of intense study (Hubbard, 2000). The ameloblasts are connected to each other via junctions that are found at both the distal and proximal ends of the cells, thus forming a continuous cell layer enclosing the developing enamel. Minerals, which are supplied via the blood, must somehow pass this ameloblast layer in order to be incorporated into the developing enamel.

In general, when nutrients must pass a cell layer, two possible pathways exist: *between* the cells (paracellular) and *through* the cells themselves (transcellular). The paracellular pathway is a form of passive transport, whereby molecules or ions are moved via diffusion following concentration gradients. In transcellular transport minerals must cross the cell membranes, which can take place via diffusion (either simple diffusion, or facilitated diffusion, which involves specific transport proteins) or via carrier-mediated active transport. The latter type of transport requires metabolic energy, such as ATP, and molecules or ions can be transported *against* a concentration gradient. In the case of active transport, the cells can exert some degree of control with respect to ion transfer. For example, the transport of certain elements can be completely or partially inhibited.

Some evidence with regard to the nature of ion transport comes from experimental work with radioactive isotopes using animal models. During the matrix deposition stage, when there is only a limited degree of mineralization, the amount of Ca entering the forming enamel appears to be controlled by the ameloblast layer. Although some Ca seems to enter the enamel via the paracellular pathway, most of the Ca is thought to move through the ameloblast cells (Hubbard, 2000). Phosphate ions have been shown to move freely through the paracellular pathway, but they follow the movement of calcium ions, which is controlled by the ameloblasts (Bawden & Wennberg, 1979; Takano *et al.*, 1983). Other studies have suggested that the integrity and/or the metabolic activity of the cells of the enamel organ is required to control movement of ions into the enamel (Bawden & Wennberg, 1979; Bawden *et al.*, 1982). Metabolic inhibition or removal of the enamel organ resulted in enhanced uptake of some elements, such as Cd, Fe, Sr, V and Zn.

It was found that selenium (Se) was taken up only during matrix deposition, and was lost during the maturation stage (Bawden *et al.*, 1982), suggesting this element is bound to, or incorporated into, proteins, which are removed during enamel maturation (see below). Copper and Fe are also often found in association with proteins (De Renzis *et al.*, 1969). Although the majority of Pb ions will take up Ca positions in the hydroxyapatite lattice, a portion may occur in the organic fraction (Kato, 1983). Ung Bao *et al.* (1990) suggest that Mn is also associated with the 'aqueous-organic' phase.

Enamel maturation

Once the enamel has reached its maximum thickness *locally*, the ameloblasts will differentiate again and enter the maturation stage (Moss-Salentijn *et al.*, 1997). This means that cuspal enamel (which was formed first) will already be undergoing maturation, while further down the crown recently differentiated ameloblasts will have just begun the first matrix secretion. During the maturation stage, the ameloblasts go through a series of cyclical changes as they switch between alternating states of supplying additional minerals to, and withdrawing water and proteins from, the enamel (Boyde, 1989; Ten Cate, 1994). These cyclical changes in ameloblast behaviour represent one of the least understood aspects of amelogenesis, and the details of the maturation process still await clarification (e.g., Boyde, 1989; Bonar *et al.*, 1991; Cuisinier *et al.*, 1992). Nevertheless, several models have been proposed, which can be classified into two groups: the *one-step theory* and the *two-step theory* (Suga, 1983).

The first model describes the process of mineralization as a more or less gradual but continuous process, starting with the beginning of matrix deposition and *continuing on* until the end of mineralization (e.g., Crabb & Darling, 1962; Deutsch & Pe'er, 1982). Initially the matrix is only partially mineralized, except for the highly mineralized innermost layer close to the dentine. Mineralization then spreads gradually from this inner layer outward to the enamel surface. The gradual mineralization of the volume of enamel follows a gradient from the tip of the crown (where the ameloblasts first begin the process of matrix secretion) to the more cervical regions (where the ameloblasts mature *later*, and thus begin the process of matrix secretion *later*). The final stage of maturation follows a similar gradient and consists of the mineralization of the outer layers of enamel, which thereby attain the highest degree of mineralization (Fig. 2.6).

An alternative model, of which Suga is the main proponent (e.g., 1982, 1983, 1989), describes the process as divided into at least two stages instead of one. The first stage is that of matrix deposition and is identical to the one described in the first model; the matrix is partially mineralized apart from the highly mineralized innermost layer at the EDJ. The second, *separate* stage is the maturation stage, which starts *at the end of* matrix formation and continues until the complete thickness of enamel has mineralized completely. According to this model, the maturation stage is characterized by a further subdivision into three separate phases. A 'wave of mineralization' is envisaged as sweeping back and forth several times between the outer and inner enamel layers (Fig. 2.7). The first phase begins at the surface and progresses inward toward the thin but highly mineralized inner layer. On reaching the innermost layer, the wave reflects and proceeds in the opposite (*i.e.*, outward) direction (second phase). The third and final phase consists of a heavy increase in mineralization of the outermost enamel. According to Suga, during the maturation stage several different 'layers' can be recognized from inner to outer enamel, each with a distinct degree and gradient of mineralization. These processes have been observed in various animal species, using a variety of techniques such as microradiography combined with tetracycline labelling, autoradiography and histochemistry. It has been proposed that this basic pattern of progressive mineralization is fundamentally the same for the enamel of all mammals (Suga, 1983).

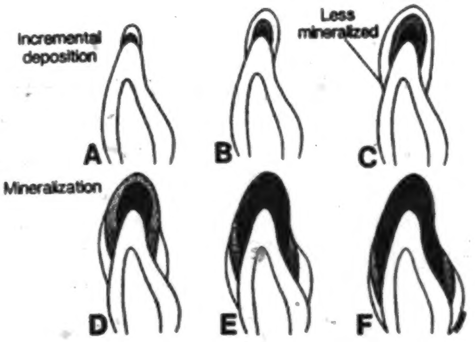

Fig. 2.6: Simplified model of enamel mineralization:
(A) Initial enamel is formed.
(B) Calcification of the first-formed enamel, further deposition of matrix.
(C-D) Formation of further increments and calcification of previously deposited matrix.
(E-F) Matrix is formed at the sides and cervical area of the crown (Reprinted with permission from Avery, 1992)

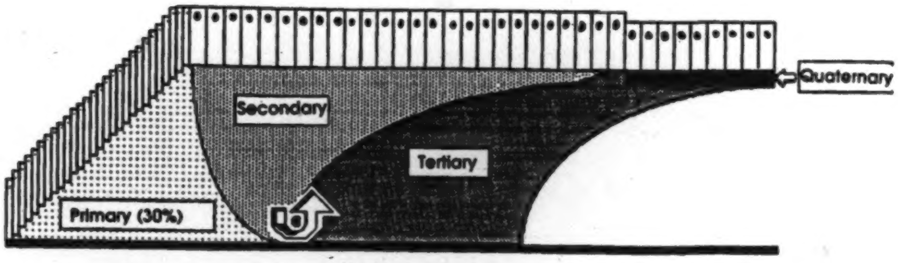

Fig. 2.7: Model of enamel mineralization according to Suga. The diagram represents the various stages of a single ameloblast as it moves outward from the EDJ (at left) to form the full thickness of enamel (primary mineralization). Secondary and tertiary mineralization are represented as a wave moving from the outer enamel to the inner enamel regions, and then bouncing back towards the enamel surface. The ameloblast then becomes shorter and the outermost layer undergoes the final stage of mineralization (Reprinted with permission from Ten Cate, 1998).

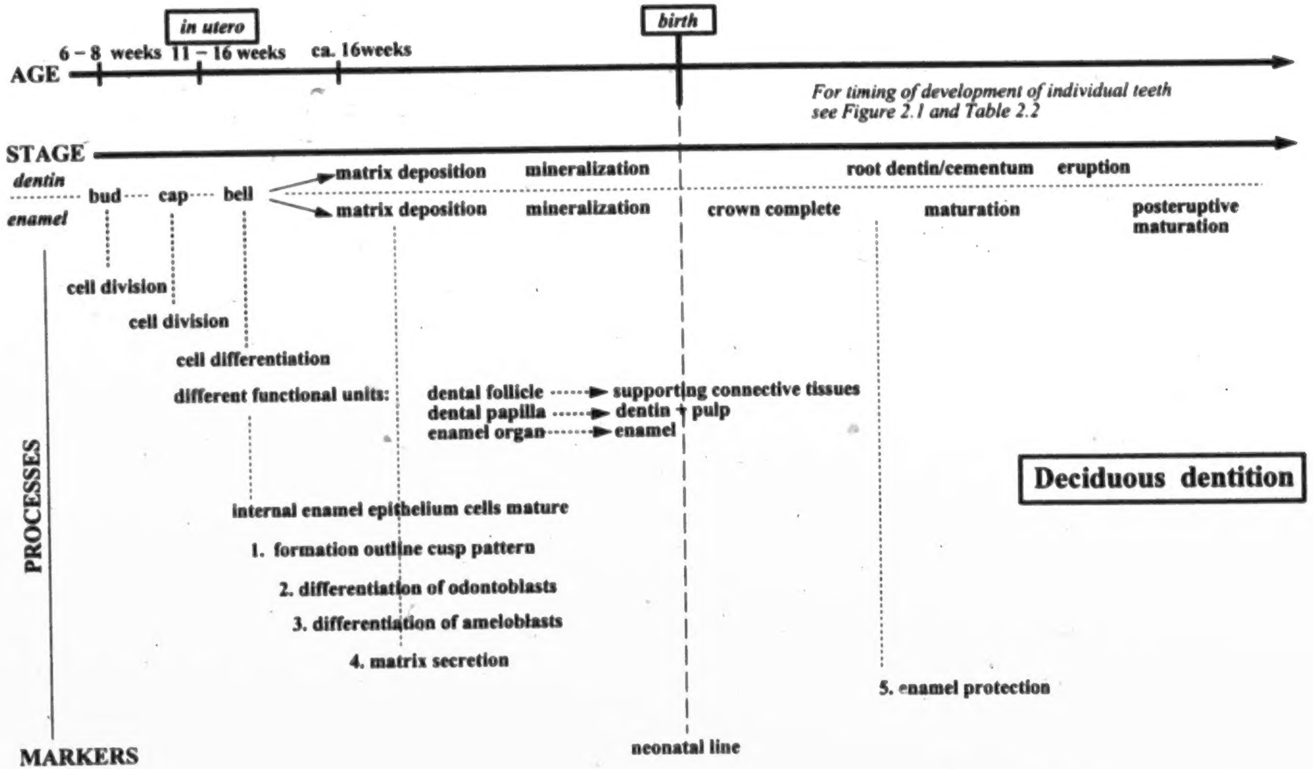
in utero
birth
AGE
6 – 8 weeks
11 – 16 weeks
ca. 16weeks
For timing of development of individual teeth see Figure 2.1 and Table 2.2
STAGE
dentin
enamel
bud
cap
bell
matrix deposition
mineralization
root dentin/cementum
eruption
matrix deposition
mineralization
crown complete
maturation
posteruptive maturation
PROCESSES
cell division
cell division
cell differentiation
different functional units:
dental follicle
supporting connective tissues
dental papilla
dentin + pulp
enamel organ
enamel
Deciduous dentition
internal enamel epithelium cells mature
1. formation outline cusp pattern
2. differentiation of odontoblasts
3. differentiation of ameloblasts
4. matrix secretion
5. enamel protection
MARKERS
neonatal line

Fig. 2.8: Diagrammatic representation of the sequence and timing of dental developmental processes for the deciduous dentition.

It is difficult to determine the time frame involved in the matrix secretion and maturation stages, as this is rarely explicitly discussed in the literature (Fig. 2.8). Apparently, the degree to which matrix formation occurs separately from enamel maturation differs between species, sites, and between deciduous and permanent tooth enamel (A. Boyde, *pers. comm.*). Rosser *et al.* (1967) found that the relative Ca concentration in developing human third molar enamel reaches a relatively high degree of mineralization close to the secretory front. This would suggest that the increase in mineralization closely follows the matrix deposition stage, which does not appear to be in agreement with the model proposed by Suga.

According to Smith (1998), the maturation stage can take as long as 2/3 of the total crown formation time. As mentioned above, the maturation process starts later for the more cervically located ameloblasts compared to those in the cuspal region. However, because the enamel in the cervical area is so much thinner than cuspal enamel, and because enamel maturation is assumed to occur fairly linearly over time, the enamel close to the cervical area will be fully matured *with only a very slight time delay* relative to the thick cuspal enamel (Smith, 1998). This would suggest that different regions of the crown complete the maturation process more or less simultaneously.

Mineral transport during the maturation stage

The immature, partially mineralized enamel is sometimes described as 'soft and cheesy' (Bonar *et al.*, 1991) with a high concentration of protein (Deutsch & Pe'er, 1982). At this point of development, the enamel is still relatively porous and ions can probably move through it by diffusion. This process will become more difficult as the degree of mineralization of enamel increases from about 30% to almost 97-98 % during maturation.

As described above, the maturation stage ameloblasts alternate in a cyclical manner between states of depositing additional minerals to, and withdrawing water and proteins from, the forming enamel. During these two distinct states, the ameloblasts show a very specific morphology. Minerals appear to be added when the cells have a 'ruffled border', while water and proteins are withdrawn when the cells have a smooth border. This shift in morphology can take place very rapidly and the mechanisms involved are not understood yet (Smith, 1998). The ameloblasts can control the junctions between the cells at the proximal and distal borders separately, making them either 'tight' or 'leaky'. In this way, open channels for transport can be created between neighbouring cells allowing for the rapid, passive movement of ions. Conversely, tight junctions create some kind of 'barrier' which forces transport *through* the ameloblasts, which can then control entry of ions into the enamel (e.g., Takano *et al.*, 1983; Hubbard, 2000).

Clearly, the bulk of the minerals that are found in mature enamel are deposited during maturation. However, it is important to point out that those elements that occur in association with proteins may actually disappear during this stage, such as Se, Cu and (possibly) Mn. Such elements may be relatively abundant in the initial matrix, which is rich in proteins. Because the enamel proteins are gradually (and almost completely) resorbed during maturation, the concentrations of these elements may be substantially reduced in mature enamel.

Whereas some elements are preferentially built into enamel during the matrix formation stage, others are mainly incorporated during the maturation stage, such as was found for Mo in rats (Bawden & Hammarström, 1976). Strontium is readily incorporated into maturing enamel and gradually accumulates throughout the thickness of enamel. After 72 hours the distribution of the isotopic tracer was found to be fairly uniform (Olsen & Jonsen, 1979).

Eruption and post-eruptive maturation

After completing the maturation process, the ameloblasts tightly secure themselves to the enamel surface in order to protect it. The bulk of the tooth is further formed by the extending odontoblasts, adding layer upon layer of dentine and creating the root of the tooth. Beyond the cervical edge of the enamel, the dentine is covered by a layer of cementum. Both dentine and cementum formation continue throughout the life of the individual. As the root is developing, the tooth gradually grows out of the protective bony crypt. The protective layer formed by the ameloblasts is worn away when the tooth finally erupts into the oral cavity and becomes functional (Avery, 1992; Ten Cate, 1994).

Although enamel maturation appears to be largely completed prior to eruption, enamel composition is known to change after eruption, but on a much smaller scale and without the mediating effect of the ameloblasts. Post-eruptive maturation generally only involves the outermost layers of enamel (30-50 µm), and consists of elemental exchange (mainly by diffusion) with the oral environment (Curzon & Cutress, 1983).

Steadman *et al.* (1958) suggest that Sr is mainly laid down before eruption, with no further post-eruptive uptake. However, Cutress (1972) and Little & Barrett (1976) found a Sr-gradient from the enamel surface inward, indicating that the element may in fact be acquired post-eruptively. Like Sr, most of the Zn appears to be deposited prior to eruption of the tooth, and post-eruptive deposition is probably irregular (Brudevold *et al.*, 1963). Because drinking water usually contains significant amounts of Fe, this element shows continuous post-eruptive uptake (Curzon, 1983). Several studies have indicated that Cu is quite readily taken up by the enamel surface, where it can accumulate in appreciable amounts. Enamel that has been altered by a carious lesion appears to be particularly accessible (Little & Steadman, 1966). Lead is also taken up post-eruptively, and levels will vary according to the degree of environmental exposure. The Pb-profiles show a very steep gradient from the enamel surface inward, and they are distinctly different between individual teeth (Malik & Fremlin, 1974; Brudevold *et al.*, 1977).

Enamel structure

Mature enamel is a highly complex material, with an intricate 3-dimensional structure. This structure arises during the matrix formation of enamel, when the ameloblasts follow their path from the EDJ outward until the crown reaches its complete thickness. The structural characteristics are not obliterated by the increase in mineralization during maturation and are preserved in the completed tooth. The most basic unit of enamel appears to be the prism (Jenkins, 1978; Boyde 1989). The prism is a hexagonal structure which in turn is composed of individual crystallites, themselves hexagonal in shape. The innermost layer of enamel is characterized by a random packing of enamel crystallites, and is termed *structureless (*or *prismless) enamel.*

When the ameloblasts have moved some distance away from the EDJ, the cells begin to develop a pointed extension called the *Tomes' process* (Fig. 2.9), which is involved in secretion. As soon as the Tomes' processes have developed, the orientation of the enamel crystallites becomes much more organized. Near the tip of the Tomes' process the crystals have an orientation different from those near the surface of the process close to the body of the cell (Fig. 2.10). This gives the enamel its characteristic structure of enamel prisms and interprismatic regions[1]: the interprismatic enamel is formed by the crystallites excreted from the regions close to the bodies of several adjacent ameloblasts, whereas the prismatic enamel consists of those crystallites that form near the surface of the Tomes' process. The interprismatic enamel is secreted first and thus forms the 'walls' into which the Tomes' process fits, creating the typical 'keyhole'-pattern of enamel (Fig. 2.10). When the formation of the whole volume of enamel is almost completed by the ameloblasts, the Tomes' processes largely disappear, so that the outermost layers of enamel are again structureless (Jordan & Abrams, 1992; Ten Cate, 1994). This outer enamel layer is thicker in deciduous teeth than in permanent teeth (Boyde, 1989).

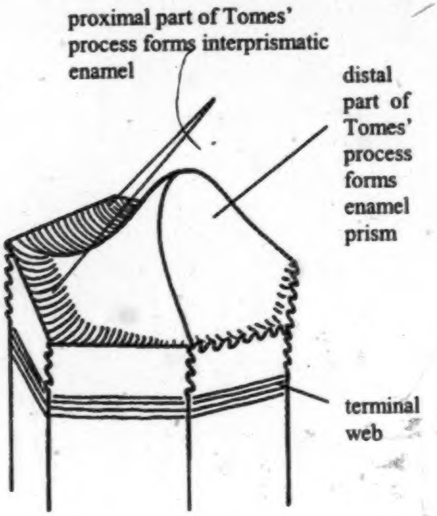
proximal part of Tomes' process forms interprismatic enamel
distal part of Tomes' process forms enamel prism
terminal web

Fig. 2.9: Ameloblast with Tomes' process. The distal part projects into the enamel and forms the enamel prism. The proximal part rests on the enamel surface and forms the interprismatic enamel (Adapted from Avery, 1994).

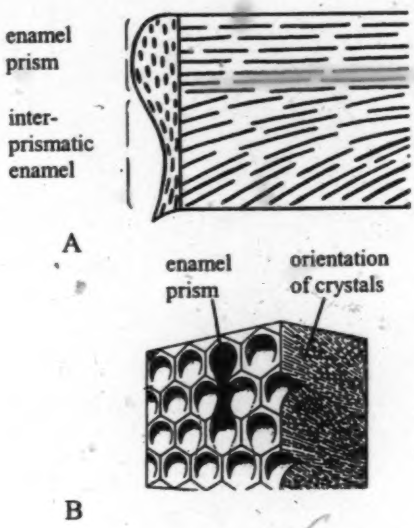
enamel prism
inter-prismatic enamel
A
enamel prism
orientation of crystals
B

Fig. 2.10. A: Schematic representation of the crystal orientation in prismatic and interprismatic enamel. B: Diagram of the interface between the enamel prisms and the ameloblasts. The shaded area shows the characteristic 'keyhole' pattern of the enamel prisms (Adapted from Avery, 1994).

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[1] The terms which have traditionally been applied to describe this structure are *prisms* and *interprismatic regions*. Since the structures are not in any way prismatic, Ten Cate (1994) considers this terminology confusing and inappropriate and prefers the use of *rod* and *interrod*. However, since most researchers still use the traditional terms, they will be used here as well.

When the ameloblasts move outward from the EDJ, they gradually increase in diameter. The total surface of the crown is therefore larger than the 'surface' area at the EDJ. In addition, while they move outward, the ameloblasts move with respect to their neighbours both vertically and horizontally (Osborn, 1973; Boyde, 1989). This pattern gives the enamel both strength and flexibility. When viewed under a microscope with polarized light a set of alternating light and dark bands, known as Hunter-Schreger bands, become visible. These bands arise due to the different orientations of the prisms with respect to the polarized light.

In addition to Hunter-Schreger bands, several other banding patterns that are often described as *incremental lines* are visible in enamel (Fig. 2.11). There are several indications that they arise due to a rhythmic deposition of enamel matrix (see Dean, 1987; FitzGerald, 1998 for reviews). *Microstriations* have been associated with a daily increment in enamel formation, which is also referred to as a circadian rhythm. Based on the average number of 7-8 microstriations, which are found between two consecutive *Striations of Retzius*, the latter are thought to represent a circaseptan rhythm, *i.e.*, a 'weekly' interval of enamel formation (Risnes, 1986; Dean, 1987; Dean *et al.*, 1993). The number of microstriations between Retzius lines has been found to vary from 6-10 (Dean, 1987) or even 4-11 (Huda & Bowman, 1994). However, the number appears to be constant in the teeth of one individual (FitzGerald, 1998).

Initially, the Retzius lines form superimposed domes in the cuspal/incisal area of the crown. This apical enamel is referred to as 'appositional' enamel. Once the full thickness of enamel in this area has been deposited, enamel formation continues with the deposition of 'imbricational' enamel, which is best described as sleeves of enamel that surround the tooth (Fig. 2.11). In contrast to appositional enamel, where the Retzius lines are 'buried', in imbricational enamel they reach to the surface where they are visible as *perikymata*.

Another line, which can be found in all deciduous teeth and often in the first permanent molars, is the *neonatal line* (Massler *et al.*, 1941; Weber & Eisenmann, 1971; Whittaker & Richards, 1978; Skinner & Dupras, 1993). This line appears as a more defined Retzius line, and represents a disturbance in enamel formation resulting from the physiological stress associated with birth.

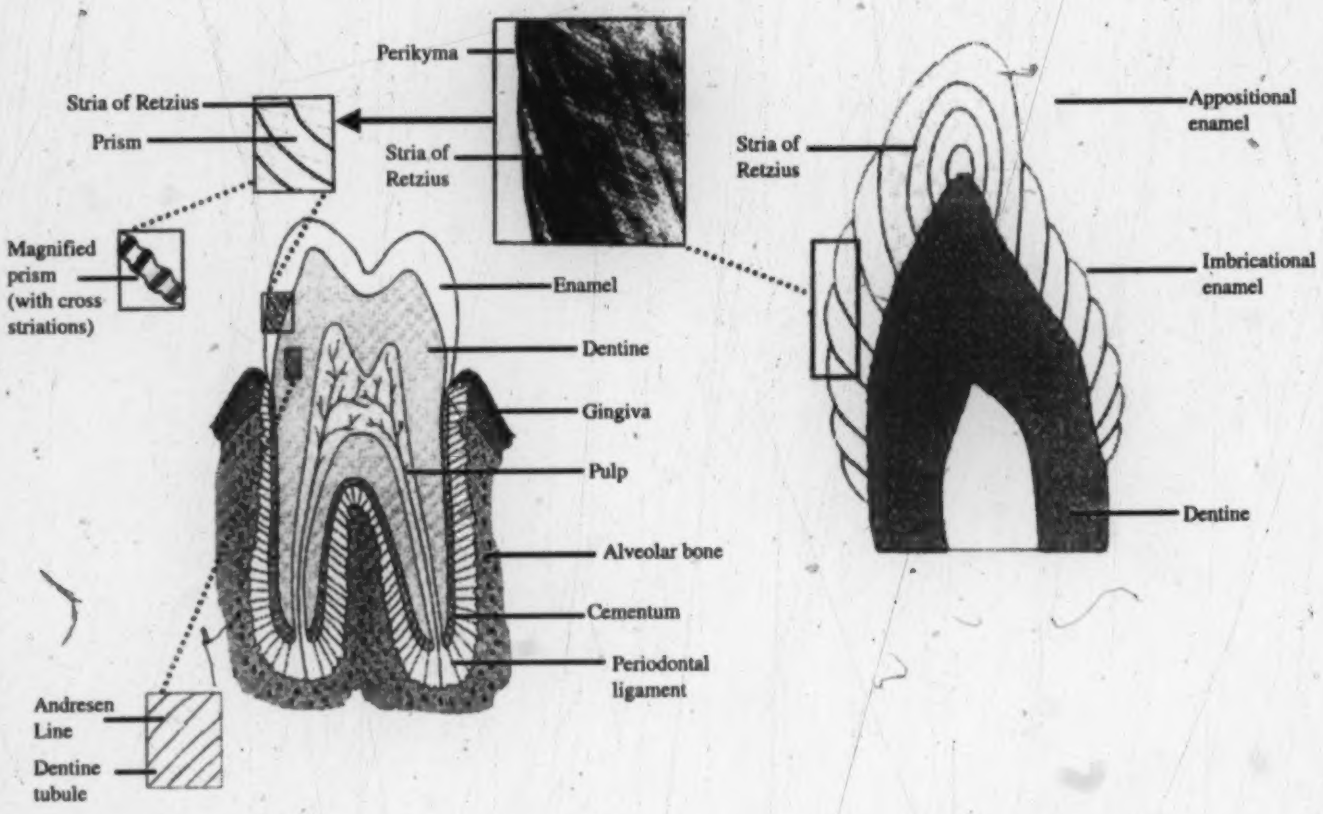
Perikyma
Stria of Retzius
Prism
Stria of Retzius
Magnified prism (with cross striations)
Enamel
Dentine
Gingiva
Pulp
Alveolar bone
Cementum
Periodontal ligament
Andresen Line
Dentine tubule
Stria of Retzius
Appositional enamel
Imbricational enamel
Dentine

Fig. 2.11: Diagrams showing the most important tooth structures and microstructures of enamel and dentine discussed in the text. N.B.: cross striations= microstriations. (Adapted from FitzGerald & Rose, 2000).

The regular incremental lines, and the neonatal line, which can be used as a marker for birth, have been used to estimate age at death for juvenile modern humans and fossil hominids (e.g., Boyde, 1963; Huda & Bowman, 1995), and for the calculation of crown formation times (e.g., Beynon *et al.*, 1998; Reid *et al.*, 1998). There has been considerable debate about the use of the incremental lines for these applications, with some researchers arguing that there is insufficient evidence for the regular periodicity of the microstriations and Retzius lines (see e.g. the special issues of the *American Journal of Physical Anthropology* 86(2), 1991, and the *Journal of Human Evolution* 35(4), 1998). It appears however, that a consensus has been reached and both types of markers are more or less accepted by the majority of the research community as representing regular time intervals of circaseptan and circadian enamel secretion, respectively (FitzGerald, 1998). We will return to these aspects at the end of Chapter 3.

Enamel composition

Enamel consists mainly of hydroxyapatite crystals. The remainder, about 4 wt%, is a water-filled protein and lipid matrix (Curzon & Featherstone, 1983). The major components of hydroxyapatite are calcium, phosphorus and the hydroxyl ion. The average Ca content is around 36 wt%, while P, mostly in the form of phosphate, is present at levels between 16-18 wt%. Another major constituent is carbonate, which is present at ca. 5% in the bulk of enamel, but is reduced to around 2-3% in the outermost layer (Curzon & Featherstone, 1983).

During the formation of enamel, several ions can substitute for each of the major components. For example, Ba^{2+}, Sr^{2+}, and Pb^{2+} can substitute for Ca^{2+}. Carbonate ions can replace PO_4^{3-} and F^- and Cl^- can substitute for the hydroxyl ion (Table 2.4). In addition, ions that are either too large to enter the apatite lattice or which have an inappropriate charge may still be adsorbed onto the crystal surface in the hydration layer that surrounds the crystals.

The greater the level of substitutions, the more the composition of the hydroxyapatite will deviate from ideal, stoichiometric apatite. From a chemical perspective the various substituting ions in enamel can be considered as impurities.

Table 2.4: Lattice substitutions and vacancies for hydroxyapatite (From Williams & Elliot, 1989).

Ion(s) to be replaced	Replacement
PO_4^{3-}	AsO_4^{3-}
	HPO_4^{2-} , CO_3^{2-} (both limited)
Ca^{2+}	Sr^{2+}, Ba^{2+}, Pb^{2+}
	Na^+, H_2O, vacant site (all limited)
	K^+, Mg^{2+} (both very limited)
OH^-	F^-, Cl^-, Br^-, I^-
	H_2O, vacant site (both limited)
$2OH^-$	CO_3^{2-}, O^{2-}

Although some of these ions may fit perfectly within the crystal lattice, ions with an inappropriate size or charge may cause distortions. In addition, there may be gaps in the lattice (called "vacancies") where ions are missing. Both the substitutions and vacancies affect the structural and chemical properties of the hydroxyapatite, such as, for example, its resistance to dissolution in acids (*cf.* the caries process). The fluoride ion, which can replace the hydroxyl ion, is known to decrease the solubility of hydroxyapatite (Van der Lugt *et al.*, 1970; Jenkins, 1978; LeGeros & Tung, 1983), whereas Mg and carbonate have been shown to result in an increased solubility (Jenkins, 1978; Jordan & Abrams, 1992).

Losee *et al.* (1974b) screened human enamel, from individuals under 20 years of age and from varying geographic areas, for 69 minor elements and found that at least 41 elements regularly occur in enamel. It appeared that some sort of selection process takes place, such that elements with an atomic number over 60 are excluded from incorporation into the apatite lattice, with the exception of Pb (atomic number 82). In fact, Sr (atomic number 38) seems to be one of the heaviest elements regularly occurring in enamel. Table 2.5, based on studies discussed in Curzon & Cutress (1983), provides an overview of the trace elements that have been detected in the enamel and dentine from permanent and deciduous teeth. It must be noted that there is considerable variation in reported averages between these studies, which can to some extent be explained by differences in analytical techniques and sample preparation procedures (Curzon & Cutress, 1983). In addition, several elements that are listed as 'not detected' have, in fact, been detected in some cases. For example, enamel from older individuals can contain trace elements from the environment that have accumulated to measurable levels. In the enamel of younger individuals, such elements are usually not yet present in sufficient amounts. Reporting of elements also depends on the analytical instrumentation used, and analytical sensitivity has improved considerably in the period since Curzon and Cutress published their summary.

Table 2.5: Overview of trace elements detected in enamel from permanent and deciduous teeth (From tables 3-1, 3-11 and 3-13 in Curzon & Cutress, 1983). Trace elements used in this study appear in bold.

Concentration range (ppm)	**Elements in permanent teeth**		**Elements in deciduous teeth**	
	enamel	**dentine**	**enamel**	**dentine**
> 1000	Na, Cl, Mg	Na, Mg	Na, Cl, Mg	Na, Mg
100-1000	K, S, **Zn**, Si, **Sr**, F	Cl, **Zn**, **Sr**, Si, K, **Ba**	K, Al, **Zn**	Cl, **Zn**, Al, K
10-100	**Fe**, Al, **Pb**, B, **Ba**	**Pb**, Br, **Fe**, Al, Rb	**Sr**, **Pb**, **Ba**, **Cu**, **Ni**, **Ti**, **Fe**	**Pb**
1-10	**Cu**, Rb, Br, **Mo**, Cd, I, Ti, **Mn**, Cr, Sn	W, **Cu**, Co, Cr, Ag, Sn, I	**Mn**, Se, Cd, Si, **Mo**	**Cu**, **Mn**, **Fe**, Se
0.1-0.9	Ni, Li, Ag, Nb, Se, Be, Zr, Co, W, Sb, Hg	**Mn**, Sb, Se		
< 0.1	As, Cs, V, Au, La, Ce, Pr, Nd, Sm, Tb, Y	Au, Cd, Pt		
Not detected	Sc, Ga, Ge, Ru, Pd, In, Te, Eu, Gd, Dy, Ho, Er, Tm, Lu, Hf, Ta, Re, Os, Ir, Pt, Tl, Bi, Rh			

The values in Table 2.5 are only a general indication of the average levels at which the various elements occur in enamel and dentine. They do not reflect the variability (and extreme values) that has been found between or within the teeth from one individual, or between the teeth from different populations.

Based on the studies discussed by Curzon & Cutress (1983), and other studies discussed here and in Chapter 1, the factors which contribute to the variation in enamel composition can be summarized as below (see also Jenkins, 1978; Sachs, 1978):

1. Diet and environment. Variation in dental composition between individuals or populations living in different geographic areas is the result of local geochemistry, which translates into the composition of locally produced/obtained food and drinking water. Examples of trace elements showing geographic variation include Cu, Fe, Mn, Sr, Mo, Zn and, possibly, Ba. There are indications that Mn is increasingly incorporated into enamel with increasing dietary Mn-intake (Mansell & Hendershot, 1960), as appears to be the case for Ba (Healy & Ludwig, 1968) and Mo (Mills & Davis, 1987).

2. Food choice/preparation. Certain elements may occur in high concentrations in particular foods depending on the preparation method used. An example is the practice of adding the ash of green plants during the preparation of cornmeal products by Hopi as mentioned in Chapter 1 (Kuhnlein & Calloway, 1977).

3. Type of tooth[2]. Inter-tooth variation within the dentition (both deciduous and permanent) of a single individual most likely reflects the oral environment at the time of development, as affected by nutritional status and dietary habits. For the deciduous dentition, maternal nutritional status will contribute to prenatal developmental circumstances (see Chapter 3). There can also be differences between the deciduous and permanent teeth of the same individual. For example, Cutress (1972) found lower concentrations of Sr, and lower Zn levels at the tooth surfaces, in deciduous teeth than in permanent teeth.

4. Position in tooth. Intra-tooth variation; examples include the observed concentration profiles for Mn, Zn, Pb and possibly Sr (see also Chapter 1). The results for Sr are not consistent, with some researchers reporting an even distribution across enamel (Steadman

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[2] Jenkins (1978) mentions that no differences between tooth types have been found between the sexes. However, many of the studies have been carried out on populations in modern industrialized countries where both sexes have roughly similar diets. Therefore, it would be interesting to take samples from populations where sex/gender-based differences in nutrition exist from early infancy onwards. It is important to keep in mind that anthropologists and dentists generally have a different set of questions while looking at the same material.

et al., 1958), and others reporting a higher concentration at the surface (Cutress, 1972; Little & Barrett, 1976).

This variation is probably the result of physical processes such as diffusion in the outermost enamel layers. Because diffusion cannot play a significant role at deeper levels, differences in elemental concentrations in deeper enamel, from the tip of the crown to the cemento-enamel junction, are most likely related to changes in environmental conditions (e.g., nutritional status) at the time of calcification.

Because caries lesions develop in the outermost (30-50 μm) layers of enamel, variations in trace element levels with depth have received much attention in the clinical literature. However, it is important to note that, from our perspective, the outer enamel layer is where we most likely will not be able to record biogenic levels: the outer layers are prone to ionic exchange with the burial environment.

5. Age. This refers to intra-tooth variation in composition due to post-eruptive uptake of elements with increasing age. With age, the outer layers become somewhat less permeable due to increased mineralization. Some elements may accumulate in this outer region. Diffusion is the primary mechanism, mediated by the physical and chemical characteristics of the elements. Since permanent teeth are exposed to the oral environment for much longer periods, this factor is more important for the permanent dentition than for the deciduous dentition.

Strontium concentrations are reported as showing either no change or a decrease with age (Steadman *et al.*, 1958; Derise & Ritchey, 1974). In general, the Zn levels in unerupted teeth are lower than in erupted teeth. However, there does not appear to be a gradual increase in Zn concentrations with age (Brudevold *et al.*, 1963). Like Zn, Pb shows a decrease from outer to inner enamel in both erupted and unerupted teeth (Brudevold & Steadman, 1956), and the element is taken up into outer enamel layers throughout life. Both unerupted and newly erupted teeth have been reported as containing no Mo (Curzon *et al.*, 1971), although this can probably to some extent be explained by the fact that Mo levels in enamel are often below detection limits of various analytical techniques.

6. Carious vs. non-carious teeth. This factor refers to within-tooth variation on a microscale (local dissolution, *i.e.* demineralization). The cause of this variation is not fully understood at present, in part due to the problems inherent in the study of caries. It is impossible to sample enamel prior to the development of a carious lesion, because one cannot predict where a lesion will develop. Once a carious lesion has developed, it is not possible to determine its original composition since the local demineralization processes will already have produced changes in the local composition (Jenkins, 1978). Copper appears to be taken up in appreciable amounts in carious enamel (Little & Steadman, 1966; De Renzis *et al.*, 1969).

Tabel 2.6: Summary of the information available for the trace elements used in this study (based on tables 3-8 and 3-9 in Curzon & Cutress (1983), and studies discussed in the text).

Trace elements		Concentration range in enamel	Associated with organic or inorganic	Variability with			Erupted vs. unerupted	Deciduous vs. permanent	Timing of incorporation
				depth from surface	age	geographic region			
Essential	Cu	1-10 ppm wide variation	Organic (partly?)	No apparent gradient	No change/ decrease	Yes			Mostly during matrix formation but also surface accumulation; altered (carious) enamel very receptive to Cu
	Fe	10-100 ppm wide variation	Partly organic?		No change?	Yes			Not during maturation (in rats); continuous post-eruptive uptake
	Mn	1-10 ppm	Organic?	Higher at surface	No change/ decrease	Yes			Timing? ; increased uptake when dietary Mn levels higher
	Mo	1-10 ppm generally low	Ca-position	No apparent gradient	Increase?	Possible; mainly related to soil	Unerupted and newly erupted teeth contain no Mo		Not during matrix formation; Taken up during final stage of mineralization; increased uptake when dietary Mo levels higher
	V	< 0.1 ppm	Phosphate position		No change?				
	Zn	100-1000 ppm	Ca-position	Higher at surface	No change/ decrease	Yes	Decrease from surface in both erupted and non-erupted teeth; Zn conc. in unerupted lower than in erupted	Deciduous enamel lower conc. at surface than permanent enamel	Major deposition before eruption; post-eruptive uptake irregular; increased uptake when dietary Zn levels higher
Non-essential/toxic	Ba	10-100 ppm	Ca-position			? (possibly)			
	Sr	100-1000 ppm	Ca-position	Evenly distributed/ Higher at surface	No change/ decrease	Yes; mostly related to drinking water	Similar in erupted and unerupted teeth from same geographic area	Deciduous lower conc. than permanent	Mostly during maturation; no post-eruptive incorporation
	Pb	10-100 ppm	Ca-position (possibly partly organic)	Higher at surface; steep gradient; pattern specific to individual	Increase	Yes	Decrease from surface in erupted and unerupted teeth		Post-eruptive uptake; according to environmental exposure

Summary

Table 2.6 is an overview of the information about the selected trace elements presented in this chapter. Based on our current understanding of enamel formation, the following conclusions can be drawn:

- The composition of enamel from different teeth may vary according to dietary intake (food and water) and environmental exposure (reflecting local geochemistry) during formation time;
- Some elements are taken up selectively during the matrix deposition phase, while others are taken up primarily during enamel maturation. Elements which occur primarily in association with proteins may be selectively removed from enamel during maturation;
- The ameloblast layer can, to some extent, control the entry of ions into enamel, and as such can behave as a 'barrier'. The uptake of elements in enamel is therefore not necessarily proportional to the trace element levels circulating in blood plasma;
- Several trace elements show changes with depth from surface, partly due to processes during the maturation phase and partly due to post-eruptive surface uptake (also accounting for age effects);
- If Suga's model for enamel maturation is correct, there is no *direct* relationship between incremental structure and enamel composition within individual teeth, because a wave of mineralization sweeps back and forth across the full thickness of enamel several times.

In this chapter we have seen that the trace elements that can be incorporated into developing enamel, are supplied to the ameloblasts via the blood circulation. The trace element levels in blood are determined, amongst other factors, by dietary intake. In the following chapter, we will look in more detail at dietary intake of trace elements during the period of dental development.

CHAPTER 3

NUTRITION DURING THE PERIOD OF DENTAL DEVELOPMENT: A Trace Element Perspective

Introduction

In order to understand the trace element composition of enamel, we have to determine the pathways of trace elements into teeth. As discussed in the previous chapter, during the formation and subsequent maturation of enamel the ameloblasts obtain their supply of nutrients from the blood. The composition of blood is a function of nutritional status, which in turn is a function of dietary intake (food and drinking water), environmental exposure, as well as such variables as age, sex, and health of the individual.

Broadly speaking, with regard to dietary intake the period of dental development can be subdivided into 3 stages:

1) The period before birth (*in utero*)
2) The lactation period (infancy), including weaning
3) Development of an adult-like pattern of dietary intake (childhood).

Figure 3.1 is a schematic representation in which these three dietary stages are combined with aspects of dental development. The x-axis represents the age of the individual, while the y-axis represents energy requirements of the growing infant. The bold curve shows the increasing energy and nutrient requirements. The thin curve represents the fraction of these requirements provided by breast milk, while the dashed curve indicates the fraction provided by additional foods. Below the x-axis, the bars indicate the period of crown development of the primary and secondary dentition (as in Tables 2.2 and 2.3). These bars are linked to the age-axis at the points of birth, ca. 6 months (often the recommended age of introduction of complementary[1] foods, based on increased needs of the infant), and the point in time where complete cessation of breast feeding takes place.

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[1] **Complementary foods:** foods that are given in addition to breast milk; **Supplementary foods:** foods that replace breast milk, e.g. cow's milk, formula, semi-solid foods (Dettwyler & Fishman, 1992).

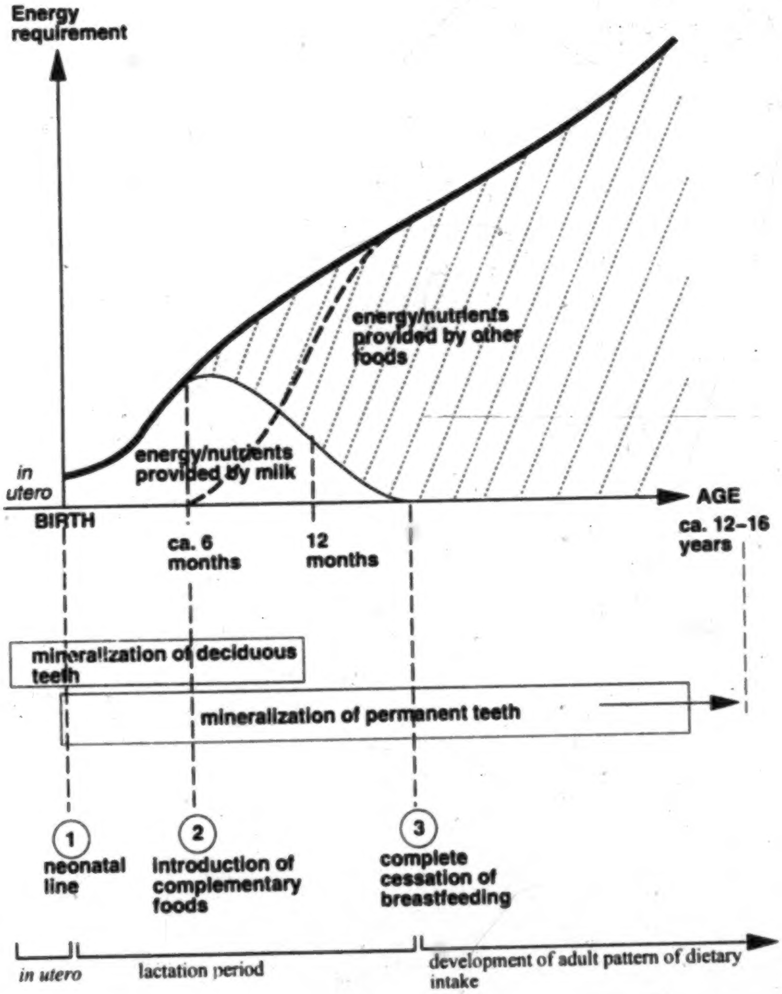
Energy requirement
in utero
BIRTH
energy/nutrients provided by milk
energy/nutrients provided by other foods
AGE
ca. 6 months
12 months
ca. 12–16 years
mineralization of deciduous teeth
mineralization of permanent teeth
1
neonatal line
2
introduction of complementary foods
3
complete cessation of breastfeeding
in utero
lactation period
development of adult pattern of dietary intake

Fig. 3.1: Schematic representation of the timing of the mineralization of deciduous and permanent tooth enamel in relation to three periods of dietary intake: the period in utero and the lactation period (both related to maternal dietary intake) and the development of an adult pattern of dietary intake following the complete cessation of breastfeeding. ***Bold curve****: the increasing energy and nutrient requirements of the developing infant;* ***thin curve****: the fraction of these requirements provided by breast milk;* ***dashed curve****: the fraction provided by additional foods.*

Each of these three points in an individual's development can provide us with a 'life history marker', indicated with 1, 2 and 3 in the figure, which could theoretically be detected in teeth:

1) Birth
2) The introduction of solid foods
3) The complete cessation of breastfeeding (sometimes this 'event' is referred to as weaning[2]; the timing will vary among populations as well as among individuals).

Whereas the first can be detected in the form of a neonatal line in histological sections of teeth that start development prior to birth, the latter two will depend on whether a chemical signature is left in the enamel.

A generalized model for the transfer of trace elements from mother to fetus/infant was presented in Chapter 1 (Fig. 1.1). In order to assess current knowledge of the relationships between maternal dietary intake and transfer of nutrients across placenta and mammary gland, as well as (infant) trace element metabolism, a literature search was carried out. Relevant publications derive from both the medical and nutritional literature. Apart from the major elements[3], Ca and P, the essential trace elements that have been included in this study are Cu, Fe, Mn, Mo, V and Zn. The non-essential/toxic elements are Ba, Sr and Pb. These trace elements have all been used in palaeodietary studies. Table 3.1 lists the functions and deficiency symptoms for each of these elements.

Publications about Fe nutrition are especially numerous, due to the high prevalence of Fe deficiency and anaemia worldwide. There has also been a longstanding interest in the elements Cu and Zn, which play essential roles in many fundamental life processes. Information about most of the selected elements could be found. This is partly due to the availability of powerful multi-element analytical techniques, such as inductively-coupled plasma mass spectrometry (ICP-MS), that are suitable for the determination of trace elements in blood, serum, human milk and other milk-based products. Vanadium is an exception in this respect because V, as well as some other trace elements, cannot be readily determined by conventional quadrupole ICP-MS (Krachler *et al.*, 1998). Partly due to these problems, and also because a specific function for V has not yet been described (Groff *et al.*, 1995), information about V with regard to transport across placenta and mammary gland was very limited.

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[2] In the literature the term 'weaning' has been used to refer to 1) the gradual process of introducing non-milk foods, or 2) the complete cessation of breastfeeding (Dettwyler & Fishman, 1992).

[3] The major elements (or macrominerals) include Ca, P, Mg, Na, K, Cl. They serve as structural components of tissues, as constituents of body fluids and are essential for the proper functioning of all cells. The major elements are abundant in the body, as opposed to the so-called trace elements, which generally comprise < 0.01% of the total body mass. A trace element is essential when deficient intake consistently results in impaired functioning which can only be prevented or cured by supplementation of the element in question (Mertz, 1981; Hambidge, 1991).

Research into many aspects of transplacental ion transport is still developing, and the following discussion can not be exhaustive. However, an attempt is made to identify the factors relevant to the development of our model, with a focus on the trace elements selected for this study.

Table 3.1: An overview of the elements used in this study, showing selected functions and deficiency symptoms.

Element	Selected Functions	Selected Deficiency Symptoms
Ca	Structural component of bones and teeth; essential in blood clotting, nerve conduction, muscle contraction, enzyme regulation, and membrane permeability	Inadequate mineralization of bone
P	Structural component of bones and teeth; development of skeletal tissue; activates many enzymes by phosphorylation; important component of RNA, DNA; constituent of phospholipids in cell membranes; functions in acid-base balance	Bone loss; hypophosphatemic rickets; (Deficiency is rare)
Cu	Essential for proper bone metabolism; activator metalloenzymes; function in inflammatory process; required for proper use of Fe	Anaemia related to Fe metabolism; skeletal demineralization; impaired immune function
Fe	Role in oxygen transport and storage; required by various enzymes in association with DNA synthesis, mitochondrial electron transport, neurotransmission	Anaemia; prolonged Fe deficiency results in anaemia accompanied by hyperplastic bone marrow
Mn	Reproductive function; bone growth; collagen formation; enzyme activator; constituent of metalloenzymes; various immune functions	Mn deficiency in humans not identified
Mo	Cofactor of metalloenzymes (redox function)	Rare
V	No specific function identified; can substitute for other metals; e.g. Zn^{2+}, Cu^{2+} and Fe^{3+} in metalloenzymes	Essentiality not firmly established
Zn	Component of many metalloenzymes related to fundamental life processes, incl. enzyme functions, gene expression, cell replication, membrane and cytoskeletal stabilization, structural role in hormones; physiological functions incl. tissue or cell growth, cell replication, bone formation, skin integrity, cell-mediated immunity, and generalized host defense	Growth retardation (nutritional dwarfism), skeletal abnormalities due to impaired development of epiphyseal cartilage, defective collagen synthesis and/or cross-linking, poor wound healing, delayed sexual maturation in children, impaired immune function and protein synthesis
Ba	No conclusive evidence for any essential function	–
Sr	No conclusive evidence for any essential function; possibly involved in calcification	–
Pb	Toxic	–

References: Nielsen (1986); Mertz (1987); Hambidge (1991); Aggett (1994); Groff *et al.*, (1995).

1. The period before birth (*in utero*)

During pregnancy, the fetus derives its nutrients from the mother via the placenta. From our perspective the following relationships are relevant: maternal dietary intake and the resulting maternal blood or serum composition; the transfer of nutrients between mother and infant across the placenta.

The placenta is responsible for transport of nutrients to the fetus, and removal of waste products from the fetal circulation back to the mother. Thus, the placenta serves as the fetus' kidney, intestine, liver and lungs (Shennan & Boyd, 1987; Shennan, 1992). As the interface between maternal and fetal circulation, the placenta's most important role is to provide the growing fetus with an environment that will ensure proper development. Although various homeostatic mechanisms work to keep the maternal blood composition relatively constant, it is thought that the placenta forms an additional barrier to protect the fetus from disturbing influences (Dancis & Springer, 1970). The environment *in utero* is extremely important because at this crucial early stage even small disturbances can seriously affect fundamental developmental processes.

Research into transplacental transport mechanisms is ongoing and many questions are as yet unanswered. One of the difficulties is that there are species differences in placental morphology and function. Therefore, although information about transport is available from experimental studies with animals, it can be difficult to assess whether the findings also apply to humans (Sibley, 1994). Primates and rodents have a discoidal haemochorial type of placenta, in which the maternal blood cells come into contact with the chorion, the outer membrane enveloping the fetus. Thus, to some extent, the rat and guinea pig placenta can serve as a model for the human placenta. Studies of human placentas can only be carried out around the time of birth. However, it is known that both morphology and transport properties of the placenta change over time in response to the increasing needs of the rapidly growing fetus (Schneider, 1996; Štulc, 1997).

Several important aspects of the transport mechanisms themselves are still unclear. The human placenta consists of several layers, one of which is the syncytiotrophoblast, a single continuous layer of fused cells (Shennan & Boyd, 1987). Transport of ions across these layers (which are referred to as the 'placental barrier') rests on the two mechanisms that have already been discussed for the ameloblast layer (Chapter 2), paracellular and transcellular transport. There is experimental evidence that both processes take place, although some authors question the possibility and nature of the paracellular transport routes, because the syncytium does not have cell boundaries (Shennan & Boyd, 1987; Shennan, 1992; Štulc, 1997).

In many cases, the total flow of nutrients to the fetus is the net result of maternal-fetal and fetal-maternal fluxes. There are indications that the fetus can excrete certain trace elements (e.g., lithium (Li), Mo, Cu and possibly Zn) via the umbilical artery. Such a mechanism does not seem to exist for Ca and Mn (Rossipal *et al.*, 2000). In contrast to most other elements, Fe is transported in maternal-fetal direction only (Van Dijk, 1988). This regulation of placental transport is believed to be controlled by fetal homeostatic mechanisms (Štulc, 1997).

In the following section the available evidence for the placental transport of the elements used in this study will be discussed systematically. It is important to keep in mind the problems experienced in this area of research, as outlined above. Studies of ion transport in humans are often based on a comparison of the composition of maternal and fetal serum[4] at birth (Schuhmacher *et al.*, 1996; Krachler *et al.*, 1999a, b). Since many trace elements are transported in the bloodstream bound to carrier proteins, serum can provide an indication of the trace element status of an individual.

Major elements: Calcium and phosphorus

Calcium and P are required in large amounts to ensure proper growth and development, especially of the skeleton (Shennan & Boyd, 1987). In all species studied, the concentration of Ca^{2+} was found to be higher in fetal plasma than in maternal plasma (Shennan & Boyd, 1987; Štulc, 1997). Transport of this ion therefore takes place against a concentration gradient (*i.e.* from the lower maternal to the higher fetal levels), requiring an active transport mechanism. There is experimental evidence, such as sensitivity of the transport process to metabolic inhibitors, that this is indeed the case (Štulc, 1997; Mughal & Husain; 1999; Krachler *et al.*, 1999a). Furthermore, placental transport of Ca^{2+} is competitively inhibited by other divalent ions, such as Ba^{2+}, Sr^{2+} and Mg^{2+} (Štulc *et al.*, 1990; Kamath *et al.*, 1992).

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[4] Serum/plasma: the clear fluid portion of blood, including many proteins, but excluding blood cells.

An active transport mechanism is also consistent with the report that fetal concentrations do not appear to be greatly affected by changes in maternal concentrations (Štulc, 1997), although it has been suggested that Ca transport may depend on maternal plasma Ca concentrations (Husain & Mughal, 1992; Mughal & Husain, 1999). There is an increase in transport rate towards the end of gestation (Care, 1991; Husain & Mughal, 1992).

Similar observations have been made for phosphorus. The fetal concentrations are higher than maternal concentrations, and transport is active, highly directional, takes place against a concentration gradient, and increases towards the end of gestation (Husain & Mughal, 1992; Štulc, 1997). Less is known about the transport mechanism for this element. It is not even known in what molecular form phosphorus is transferred to the fetus (Husain & Mughal, 1992).

Essential trace elements: Cu, Fe, Mn, Mo, V and Zn

Copper

During the last trimester, maternal serum Cu concentrations increase (Arnoud *et al.*, 1993). Compared to the serum Cu concentrations of normal adults, levels of this element in pregnant women are doubled. It appears that more Cu is retained, either through increased absorption or decreased excretion, possibly in relation to an estrogen effect (Davis & Mertz, 1987). The levels of Cu in maternal serum have been reported being as much as five times higher than those in umbilical cord serum (UCS) and colostrum (the first milk) (Krachler *et al.*, 1999a).

Even with the high Cu levels in maternal serum, the circulating levels in the fetus remain relatively low (UCS Cu levels are only about 35 % of normal adult levels) due to the fact that levels of ceruloplasmin, the major Cu-binding protein, in the serum of the fetus are low. Based on these observations, it has been suggested that copper may be 'blocked' in its transfer from mother to fetus (Krachler *et al.*, 1999a). Nevertheless, substantial stores of Cu are found in the fetal liver: as much as 50-70% of total body Cu is present in the liver at birth (Widdowson *et al.*, 1972; Casey & Hambidge, 1985; Hambidge, 1991; Lombeck & Fuchs, 1994; Krachler *et al.*, 1999a).

Interestingly, venous[5] UCS levels of Cu were higher in neonates of primiparous mothers than in others, although there was no variation between the maternal concentrations (Salmenperä *et al.*, 1986), which seems to suggest that somehow placental functioning is different in primiparous and multiparous mothers.

Iron

Whereas for most elements transport across the placenta occurs in both directions, Fe is transported to the fetus only (Van Dijk, 1988), as was mentioned above. Maternal blood Fe levels are lower than cord blood levels (e.g., Rios *et al.*, 1975; Tsuchiya *et al.*, 1984; Bertram *et al.*, 1998). Transport therefore takes place against a concentration gradient and is thought to require energy (Shennan & Boyd, 1987). The rate of transport increases rapidly towards the end of gestation (Van Dijk, 1988; Shennan & Boyd, 1987) which normally results in a relatively large 'store' of Fe in the newborn in blood haemoglobin (Mertz, 1987).

In order to meet the needs of the growing fetus, maternal Fe stores are mobilized. In addition, intestinal absorption of this element increases during pregnancy, especially during the last trimester (Van Dijk, 1988). Some clinical observations suggest that when maternal dietary intake of iron is low, mother and fetus will be competing for the limited amount of Fe. This will result in depleted maternal Fe stores (Rios *et al.*,1975) and eventually, depending on dietary Fe intake, development of maternal Fe deficiency anaemia. In turn, this may translate into poor Fe stores in the fetus (e.g., Singla *et al.*, 1978). However, other studies did not find a relation between Fe status of mother and infant (Rios *et al.*,1975), and iron stores in infants born to severely Fe deficient mothers do not appear to be dramatically reduced, which suggests that placental transport of Fe to the fetus is regulated by some long-term control mechanism (Van Dijk, 1988).

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[5] Blood reaches the fetus through the *venous* umbilical cord and returns to the mother via the *arterial* cord after the necessary nutrients have been extracted (Krachler et al., 1999b).

Manganese

The finding of markedly higher Mn concentrations in UCS compared to maternal serum suggests an active transport mechanism across the placenta for this element (Krachler *et al.*,1999a; Rossipal *et al.*, 2000). A number of possible explanations for these high serum Mn concentrations have been put forward, such as increased absorption of Mn in the immature gut, immaturity of the excretory pathways, and/or requirement of larger amounts of Mn for the still developing Mn enzyme systems during late fetal and early postnatal life (Mertz, 1987; Rükgauer *et al.*, 1997).

Despite these higher serum levels the newborn infant has no Mn stores; there are no known Mn 'storage proteins' (Mertz, 1987). Widdowson *et al.* (1972) have speculated that the bones may form the fetal 'store' of Mn.

Molybdenum

Information on this element is limited. Krachler *et al.* (1999a) found that Mo concentrations in maternal serum and UCS were very similar, suggesting that the placenta does not have any specific effect on Mo. In another study Krachler *et al.* (1999b) report that concentrations of Mo in venous sera were higher than the corresponding levels in arterial sera, which indicates that this element is taken up by the fetus. Rossipal *et al.* (2000) found similar concentrations of Mo in maternal and fetal sera. These authors also report that Mo can be excreted by the fetus via the arterial cord into the placenta in order to maintain homeostasis.

Vanadium

Vanadium is considered an essential trace element, as well as a toxic metal (Hackett & Kelman, 1983). The element has been found to pass the placental barrier, although the placenta itself appears to retain most of the vanadium. Of the small amounts that are distributed to the fetus, the highest concentrations appear in the fetal liver, intestine and kidneys. In a study with rats, concentrations of this element in fetal tissues were found to be higher than those in the tissues of newborns, possibly due to a greater affinity of V for developing tissues (Hackett & Kelman, 1983; Edel & Sabbioni, 1989).

Zinc

For Zn, similar to Mn, an active transport mechanism has been suggested in order to account for the almost double UCS Zn concentrations compared to maternal levels (Krachler *et al.*, 1999a, c; contra Tsuchiya *et al.*,1984, who reported that the Zn concentration in maternal blood was significantly higher than in cord blood).

Zn appears to be mobilized in the mother during pregnancy. A physiological decline in maternal plasma Zn concentrations has been observed, especially towards the end of pregnancy when the fetus requires increasing amounts of the element (Frković *et al.*, 1996; Krachler *et al.*, 1999a). Apart from being related to an increased fetal demand for this element, the decline in maternal plasma Zn could be due to an expansion in plasma volume or a decrease in Zn-albumin affinity (Frković *et al.*, 1996). Zinc may be stored in the fetal liver bound to metallothionein (Widdewson *et* al., 1972; Aggett, 1994).

Non-essential/toxic elements: Ba, Sr and Pb

Barium

As for Mo, there is very little data in the literature on serum concentrations of barium. However, Krachler *et al.* (1999a, c) found that Ba concentrations were significantly lower in UCS than in maternal serum.

Strontium

For Sr, strong correlations were found between the concentrations of this element in maternal and fetal sera, suggesting passive transport following a concentration gradient (Krachler *et al.*, 1999a; Rossipal *et al.*, 2000). These authors concluded that the placenta is no barrier to Sr. However, many studies have demonstrated that there is discrimination against Sr in favour of Ca at various levels in the body, including the placenta (Comar *et al.*, 1957, Comar & Wasserman, 1964; Twardock, 1967). Twardock (1967) found that in the guinea pig placental discrimination of Sr decreased gradually towards the end of pregnancy. He suggested that the same could occur in humans, since humans and guinea pigs have the same type of placenta.

The observation by Krachler *et al.* (1999b) of slightly higher Sr concentrations in venous sera compared to arterial sera seem to indicate that Sr is absorbed by the fetus;

most likely it is deposited in the developing hard tissues since Sr is known as a 'bone-seeking' element.

Lead

Krachler and associates found that UCS concentrations of the toxic element Pb were lower than the levels of the same element in the sera of older infants and adults. They suggest that the placenta may function as a barrier, protecting the developing fetus from exposure to harmful concentrations of toxic elements in maternal serum (Krachler *et al.*, 1999c). This conclusion is supported by Rossipal *et al.* (2000) who report that UCS concentrations of Pb were 50% of that of maternal sera. In contrast, the results from several other studies (Tsuchiya *et al.*, 1984; Ong *et al.*, 1985; Schuhmacher *et al.*, 1996; Bertram *et al.*, 1998) suggest that the placenta has no major filtering effect for Pb. These studies report strong correlations between maternal blood Pb levels and the concentrations of this element in umbilical cord blood.

The levels of Pb in maternal blood reflect environmental exposure, with the more exposed individuals (e.g. through occupation) showing the higher concentrations (Quarterman, 1986; Krachler *et al.*, 1999a).

The distribution of Pb in tissues shows similarities to that of Ca. Since Ca metabolism is altered during pregnancy and lactation, it has been suggested that, like Ca, Pb may also be mobilized from stores such as the mother's bone into more available pools, such as blood and plasma (Silbergeld, 1991; Bellinger, 1994; Schuhmacher *et al.*, 1996), thus increasing the exposure of the fetus to this toxic element as well.

In addition to Pb, several other heavy metals (e.g., Cd) are known to cross the placental barrier, but nothing is known about the transport mechanisms involved (Shennan & Boyd, 1987).

2. The lactation period

For our purposes, the relationships between maternal dietary intake (and resulting composition of the mother's blood/serum) and milk composition, and the transfer of nutrients between mother and infant via breast milk (across the mammary gland) are important.

Following birth, there is generally a period of exclusive breastfeeding, followed by a transitional period of mixed feeding (breast milk and complementary foods), up until the time of complete cessation of breastfeeding. The lactation period is often subdivided into three stages, based on changes in milk composition: from birth to around 4-7 days (colostrum, the first milk produced, differs substantially in composition from more mature milk); from 7-21 days (transitional milk); and from 21 days onward (mature milk) (Subcommittee on Nutrition during Lactation, 1991 - hereafter referred to as SNL, 1991). However, some authors believe that this subdivision is artificial, because the composition of breast milk is continuously changing. Neville (1991) for example, suggests that it would be better to refer to colostrum as 'early milk', and to the period after five days postpartum as 'full lactation', and stresses the importance of specifying the day at which the samples were collected.

The composition of breast milk is highly variable, not only among individuals, but also within an individual, during the whole lactation period, among different feedings, and within a single feeding (SNL, 1991). We need not be too concerned with the intra-individual variability on the scale of days or weeks, as the resolution of tooth compositional data, if indeed reflecting dietary intake, will most likely be on a longer time scale. However, it is important to keep this variability in mind when evaluating the literature. Conflicting results with regard to trace element levels in breast milk can be due to differences in, for example, the timing of taking samples during the lactation period, sample preparation methods, and analytical techniques.

Some of the individuals whose deciduous teeth were included in this study were fed infant formula rather than breast milk. Therefore, information from the literature about differences in trace element concentrations between human milk and various types of infant formula (based on cow's milk or soy) will be included. A wide variety of formulae is available, but generally formulae are made to resemble human milk as closely as possible. However, because of differences between cow's milk and human milk, for example with regard to casein and whey protein fractions, cow's milk-based formulae tend to be characterized by reduced bioavailability of many of the trace minerals (Schanler & Cheng, 1991). For this reason, most formulae have been fortified to meet the requirements of the infant. The bioavailability of many trace elements from soy-based formulae is even poorer than from cow's milk-based formulae, although Mn is naturally higher in the soy products (Sandström *et al.*, 1983; Davidson *et al.*, 1989; Hambidge, 1991).

The information from the literature with regard to trace element concentrations in milk, and transfer from mother to infant, will be presented below in a form analogous to that of the previous section.

Major elements: Calcium and phosphorus

The newborn has high nutrient requirements related to rapid growth during the first few months of life. The milk concentrations of calcium and phosphorus, the main constituents of the infant's forming and growing hard tissues, both appear to be regulated. Karra *et al.* (1988) found that Ca levels in milk varied with stage of lactation. There was an initial rise during the first 3 months after birth, followed by a decrease, and by 6 months the levels had dropped below the initial values. This pattern was found in American and Egyptian women of contrasting economic status, which led the authors to conclude that physiological mechanisms control the secretion of Ca in milk.

Calcium levels in human milk were found to be twice as high as those in maternal sera (Krachler *et al.*, 1999a). There are no indications that the Ca concentration in human milk is affected by maternal intake of this element (Feeley *et al.*, 1983a; SNL, 1991).

Very little information is available about phosphorus levels in milk. Feeley *et al.* (1983a) found a decrease in P levels with continuing lactation, and report that there was no significant correlation between maternal age, parity, previous lactation history and Ca and P concentrations in milk.

Essential trace elements: Cu, Fe, Mn, Mo, V and Zn

Copper

Maternal serum Cu levels, which increased during the last trimester, appear to return to pre-pregnancy levels in the first few weeks following birth (Schramel *et al.*, 1988). The Cu concentration in milk appears to decrease gradually as lactation progresses (Feeley *et al.*, 1983b; Dewey *et al.*, 1984; Salmenperä *et al.*, 1986). Arnaud *et al.* (1993) found that Cu levels declined but then levelled off around 3 months postpartum.

Several studies have examined the relation between milk composition and maternal dietary intake (e.g., Vuori *et al.*, 1980; Feeley *et al.*, 1983b; Casey *et al.*, 1989). Neither dietary Cu intake, nor the use of Cu supplements appears to affect the milk Cu concentrations. Results from a study by Salmenperä *et al.* (1986) suggest instead that the concentrations are individually regulated; some mothers appear to have high, and others low, Cu levels in most milk samples, independent of the concentrations in their serum. Zavaleta *et al.* (1995) propose that mammary gland uptake of Cu (and other trace elements), and subsequent secretion of these minerals in milk may be homeostatically regulated.

Feeley *et al.* (1983b) found no significant correlations between maternal age, parity, or previous lactation history and milk Cu content. In contrast, Picciano & Guthrie (1976) report higher Cu levels in milk from multiparous mothers, irrespective of whether they had breast fed or not, and higher Cu levels in older women. These authors also found considerable variation in milk Cu concentrations among women and within the same woman. It is difficult to unravel the relations between parity, lactation history and maternal age, since these factors are usually not independent of one another (Picciano & Guthrie, 1976).

Apart from the high Cu concentrations in milk shortly after birth, the breast fed infant has a relatively low dietary Cu intake. Nonetheless, the concentrations of serum Cu and ceruloplasmin show a steady increase during the first year of life (Salmenperä *et al.*, 1986). The actual concentrations of Cu in either breast milk or a supplemented formula do not appear to have an effect on plasma or serum Cu levels (Salim *et al.*, 1986; Lönnerdal & Hernell, 1994), which may indicate that these levels are maintained internally by the liver Cu stores formed *in utero* (Aggett, 1994). Towards the end of gestation, 50-70% of total body Cu is present in the fetal liver, bound to metallothionein (Widdowson *et al.*, 1972; Casey & Hambidge, 1985; Hambidge, 1991). The term infant normally has sufficient hepatic Cu stores for the first 4-6 months of life (Hambidge, 1991; Aggett, 1994). An infant with low Cu stores, for example a preterm infant, is at increased risk of Cu deficiency when dietary intake of Cu is low (Hambidge, 1991; Aggett, 1994).

Because human milk contains more Cu than cow's milk (or cow's milk-based formulas – Mertz, 1987), certain formulas may provide the infant with less than optimal amounts of this element. Furthermore, the bioavailability of Cu from formulas may be affected by a high Zn/Cu ratio (Lombeck & Fuchs, 1994), different ratios of casein/whey protein (Krachler *et al.*, 1999c), or high Fe levels (Lönnerdal & Hernell, 1994).

Iron

The literature on iron is quite extensive. Much of the interest in this element is generated by the puzzling fact that iron concentrations in human milk are much lower than infant requirements, resulting in a widespread occurrence of Fe deficiency and anaemia.

Like copper, Fe is normally stored (in haemoglobin) to a significant extent during gestation. During the first few postnatal months, Fe from dietary intake is added to these body stores. The specific nature of the food in which the Fe is contained appears to be more important than the actual amount of Fe an infant consumes, due to interfering effects of other food components on Fe absorption (Dallman, 1990).

The bioavailability of Fe in human milk is high (Saarinen *et al.*, 1977; Hertrampf *et al.*, 1986). Therefore, although the Fe concentration in milk is low, human milk is a good source for this trace element. There is a gradual decrease in the concentration of Fe in milk with ongoing lactation (Feeley *et al.*, 1983b; Arnaud *et al.*, 1993). Several months after birth, the increased demands for Fe in the rapidly growing infant will coincide with this reduced intake from breast milk. Whether the infant will be at risk of developing Fe deficiency at that point will depend on the size of the stores with which the infant was born. Saarinen *et al.* (1977) found that the Fe stores in infants who received breast milk during the first 6-7 months were greater than those in infants who were fed a cow's milk-based formula. Nonetheless, once the stores have been consumed, and dietary intake remains low, the infant is at risk of developing Fe deficiency (Dallman, 1996).

Exclusively breast fed term infants rarely develop Fe deficiency during the first 6 months of life (Murray *et al.*, 1978; Aggett *et al.*, 1989; Dallman, 1996). Pastel *et al.* (1981), in a study of Peruvian infants, concluded that the infant's Fe requirements will be met by exclusive breastfeeding for at least 9 months. Preterm and low birth weight infants, or infants born to Fe deficient or anaemic mothers will all have smaller Fe stores at birth. These infants are at risk of becoming Fe deficient after about 2-3 months and should receive Fe supplements (Lundstrom *et al.*, 1977; Iwai *et al.*, 1986; Aggett *et al.*, 1989; Chierici & Vigi, 1991).

Picciano & Guthrie (1976) found variation in milk Fe concentrations among women and within the same woman. There are no indications that maternal dietary intake of Fe, whether supplemented or not, or maternal Fe status, has an effect on the Fe levels in milk or on the Fe status of the breast fed infant (Murray *et al.*, 1978; Vuori *et al.*, 1980; Celada *et al.*, 1982; Fransson *et al.*, 1984; Siimes *et al.*, 1984).

Feeley *et al.* (1983b) found no significant correlations between maternal age, parity, or previous lactation history and milk Fe content. In contrast, Picciano & Guthrie (1976) found increased amounts of Fe in milk of multiparous women irrespective of lactation history. On the other hand, milk from older women showed lower Fe concentrations than milk from younger women.

Manganese

Manganese concentrations are higher in colostrum than in mature milk (Rossipal & Krachler, 1998). The element appears to be actively transported across the mammary gland (Rossipal *et al.*, 2000).

The Mn content of milk may be influenced by maternal dietary intake of this element (Vuori *et al.*, 1980). As well, for breast fed infants, Mn shows a correlation between intake and serum concentrations (Hambidge, 1991; Aggett, 1994). Bioavailability of Mn (which is bound to lactoferrin) from human milk is much higher than that from cow's milk (Mertz, 1987). Generally, however, serum Mn concentrations in both breast fed and formula fed infants are higher than in adults (Krachler *et al.*, 1999c). These high levels decrease by as much as 54% from their initial value during the first 6 months of life to adult values at 18 years (Rükgauer *et al.*, 1997). This is partly explained by a maturing Mn metabolism (absorption, excretion) and higher initial requirements due to the development of many of the Mn enzyme systems (Mertz, 1987; Rükgauer *et al.*, 1997).

Compared with human milk, bioavailability of Mn is lower in cow's milk (Mertz, 1987). Soy formulas, although naturally high in Mn, also have low Mn bioavailability which is probably due to the high phytate content (Hambidge, 1991). However, Davidson *et al.* (1989) found that the total amount of Mn in soy formula was high enough to compensate for this low bioavailability, resulting in the absorption of significantly higher amounts of Mn from soy formula compared to that from human milk.

Dietary intake of this trace element is usually more than adequate, and true Mn deficiency is not known to occur in humans (Casey & Hambidge, 1985; Aggett, 1994), although for infants the risk for both deficiency and toxicity is somewhat greater (Davidson *et al.*, 1989).

Molybdenum

Information about milk Mo levels in the literature is rather scarce. Krachler *et al.* (1999a) determined on average ten times higher concentrations of Mo in colostrum than in maternal serum, and the two variables showed no correlation. Molybdenum levels in colostrum were found to be six times that of mature milk. With continuing lactation, the Mo concentrations in milk gradually decrease, which suggests that the Mo content of milk is regulated in some way (Krachler *et al.*, 1998; Friel *et al.*, 1999). A WHO study (1989) reported generally low concentrations of Mo in human milk samples from different regions of the world. However, samples from the Philippines showed much higher Mo concentrations, possibly indicating regional (environmental) variation.

Serum Mo concentrations were found to be significantly higher in formula fed infants compared to breast fed infants (Krachler *et al.*, 1999c).

Vanadium

The literature does not offer much information about V in milk. Vanadium is capable of being transported across the mammary gland to the breastfeeding infant. In human milk

V is probably associated with an Fe-containing transferrin-like protein such as lactoferrin, which may be related to its biochemical role. Vanadium appears to be preferentially incorporated into developing bone (Edel & Sabbioni, 1989).

As for Mo, there may be regional (environmental) variation in V levels in milk (WHO, 1989). Samples from the Philippines and Nigeria showed high levels, whereas samples from Sweden and Hungary showed low levels. Within the same region (Zaire), there were differences in V concentrations between rural (higher) and urban (lower) milk samples.

Zinc

In comparison to Cu and Fe, Zn levels seem to be more comparable among women (Picciano & Guthrie, 1976). Concentrations of Zn in milk are initially quite high and then show a steep decline in the first months which becomes more gradual as lactation progresses (Feeley *et al.*, 1983b; Casey *et al.*, 1989; SNL, 1991; Arnaud *et al.*, 1993; Ortega *et al.*, 1997; Krachler *et al.*, 1998). This gradual decrease has been observed in Zn supplemented as well as unsupplemented women, which suggests that milk Zn levels are physiologically regulated (Karra *et al.*, 1988). A similar conclusion was drawn by Casey *et al.* (1989), who postulated that milk Zn levels are under genetic control.

Most of the evidence seems to indicate that the concentration of Zn in milk is not influenced by maternal dietary Zn intake (e.g., Vuori *et al.*, 1980; Feeley *et al.*, 1983b; studies reviewed in SNL, 1991). However, Karra *et al.* (1988) found higher Zn levels in the milk of women who took Zn supplements. Similarly, Ortega *et al.* (1997) concluded that maternal serum Zn levels during pregnancy and the levels of Zn in milk are determined by maternal dietary intake.

Ortega *et al.* (1997) found that women who had previous pregnancies had lower serum Zn concentrations than those who were pregnant for the first time. Frković *et al.* (1996) reported that the mean concentration of Zn of milk from mothers younger than 25 years was significantly higher than that of mothers older than 25 years. It is possible that observations on parity and age are not independent, in that younger women have probably had fewer pregnancies than older women. A possible explanation for this reduction in available maternal Zn may be found in the mechanisms which have been proposed to meet increased demand for Zn during lactation: apart from an increased absorption and retention, there may be a breakdown of tissue and a redistribution of Zn in the body, which may involve Zn from maternal bone (Casey *et al.*, 1989). However, Picciano & Guthrie (1976) found opposite results: increased amounts of Zn in milk from multiparous women, irrespective of lactation history, and higher Zn concentrations in milk from older women compared to that from younger women.

Infant requirements are high with rapid growth in the first 4 months after birth (Krebs & Hambidge, 1986). During this period, serum Zn levels in infants have been found to decrease, which may be related to the falling Zn levels with continued lactation (Lombeck & Fuchs, 1994). In the neonate, about 25% of total body Zn is found in the liver, which represents only a modest store (Hambidge, 1991). The liver Zn stores fall to their adult values at around the age of weaning, exposing the infant to an increasing risk of developing Zn deficiency (Aggett, 1994). Although 40% of body Zn is found in the infant's skeleton (Aggett, 1994), this does not represent a store that can be readily mobilized, as release of Zn through skeletal remodelling is a relatively slow process (Groff *et al.*, 1995). Thus, whether the infant will be able to maintain optimal levels of Zn for growth and development depends ultimately on the adequacy of dietary intake and absorption (Casey & Hambidge, 1985). Generally, the risk of Zn deficiency for full-term breast fed infants who are born with the usual liver Zn stores is quite low (SNL, 1991). However, Zn deficiency has been observed in both preterm and term infants, with male infants showing a higher frequency than females, which can be explained by the more rapid growth – and concomitantly higher Zn requirements – in male infants (Aggett, 1994).

Bioavailability of Zn from human milk is higher than that from either cow's milk or formula (Frković *et al.*, 1996). The retention of Zn from soy-formulas appears to be particularly low, possibly related to the high phytate content in soy (Chierici & Vigi, 1991).

Non-essential/toxic elements: Ba, Sr and Pb

Barium

For Ba, Krachler *et al.* (1999a) found almost five times higher concentrations in colostrum relative to maternal serum. In a different study the barium concentrations in colostrum were found to be higher than those in either the transitional or mature milk (Krachler *et al.*, 1998), which would suggest a decrease over time. However, Friel *et al.* (1999) observed no temporal trend for this element and suggested that milk Ba concentrations may be responsive to maternal diet.

The sera of formula fed infants showed higher concentrations of Ba than the sera of breast fed infants, possibly due to the higher levels of Ba in the formulas (Krachler *et al.*, 1999c).

Strontium

A selection against Sr has been observed during transport of this element across the mammary gland (Comar *et al.*, 1957; Comar & Wasserman, 1964). However, Krachler *et al.* (1999a) found that Sr concentrations in colostrum were twice as high as those in maternal sera. Concentrations of strontium in milk do not show any consistent trend with time (Friel *et al.* 1999).

Several studies have shown that the concentration of Sr in human milk is lower than that found in cow's milk (see SNL, 1991).

Lead

Like Sr and Ba, Pb is thought to substitute for Ca, and > 90% of total body Pb may be found stored in the skeletal tissues (Quarterman, 1986). This element, like cadmium (Cd) and mercury (Hg), is a true environmental pollutant with a high toxicity. Usually, concentrations found in the body reflect the extent of environmental exposure. The Pb concentrations in human milk were also found to vary in accord with exposure to environmental sources (Krachler *et al.*, 1998). As was mentioned in the previous section, the changes in Ca metabolism during pregnancy and lactation may affect the mobilization of Pb from the maternal bones (Silbergeld, 1991; Bellinger 1994; Schuhmacher *et al.*, 1996). Colostrum Pb levels were as much as four times higher than maternal sera

(Krachler *et al.*, 1999a), suggesting that there is no major filtering effect in the mammary gland to protect the infant from the harmful effects.

Concentrations of heavy metals (Pb, Hg, arsenic (As), Cd) tend to be higher in cow's milk and formulas than in human milk (SNL, 1991).

In Table 3.2 some differences in bioavailability of trace elements for the different types of formula are listed, together with a summary of the above presented information about trace element transport across placenta and mammary gland, and responsiveness of milk composition to maternal diet.

3. Pattern of adult dietary intake

For the developing fetus and breastfeeding infant, dietary intake is inextricably linked to maternal dietary intake. During the transitional phase of weaning, this link becomes weaker as the infant comes to rely less and less on its mother for nutrition. As the infant's gastro-intestinal tract is maturing, absorption, excretion and selection mechanisms (e.g., for Mn and Sr) will improve in efficiency. The adult pattern of Cu distribution among different body compartments is not achieved until late infancy (Aggett, 1994).

Following complete weaning, which may be at an age as early as a few months (or even earlier), or as late as 3 or 4 years (Dettwyler & Fishman, 1992; Dettwyler, 1995), a more adult-like pattern of food intake will develop. It is at this point that gender-based differences in diet may more strongly be expressed. However, favouring one sex (usually male) can start at birth (Saunders & Barrans, 1999). This third stage of dietary intake coincides with the development of most of the permanent dentition, especially when weaning takes place at a relatively young age.

Table 3.2: Summary of the information about trace element transport across placenta and mammary gland for each of the elements used in this study.

Element	**Transport across placenta**	**Transport across mammary gland**	**Response milk levels to maternal dietary intake**	**Bioavailability (% absorption)**		
				Human milk	**Cow's milk based formula**	**Soy milk based formula**
Ca	Active/bi-directional	Active Regulated	No			
P	Active/bi-directional	Active Regulated	?			
Cu	Filtered/blocked?	Regulated?	No/mobilized from body stores	High (50-70%)[a]	Low (5-15%)	
Fe	Active/uni-directional No regulation?	Regulated?	No?	High (50%)[b]	Low (7% from Fe-fortified)	Very low (1.7%)
Mn	Active?	Passive?	Possible	(9%)[c]	(3-6%; 1.5% if Fe-fortified)	Very low (1%); Elevated concentrations[d]
Mo	Passive?	Regulated?	?	(80%)	(80%)	Elevated concentrations[d]
V	?	?	?			
Zn	Active?	Regulated	?/mobilized from body stores	High (50-60%)[e]	Medium (30%)	Low (15%)
Ba	Filtered/blocked?	Filtered?	Possible			Elevated concentrations[d]
Sr	Filtered/blocked? Passive?	Filtered	?			
Pb	Passive?	Passive?	Environmental exposure/ mobilized from body stores		Elevated[f]	Elevated[f]

[a] Casey & Hambidge (1985); Hambidge (1991)
[b] Saarinen *et al.* 1977; Hertrampf *et al.* (1986)
[c] Hambidge (1991)
[d] Krachler *et al.* (1998)
[e] Sandström *et al.* (1983)
[f] Subcommittee on Nutrition during Lactation (1991)

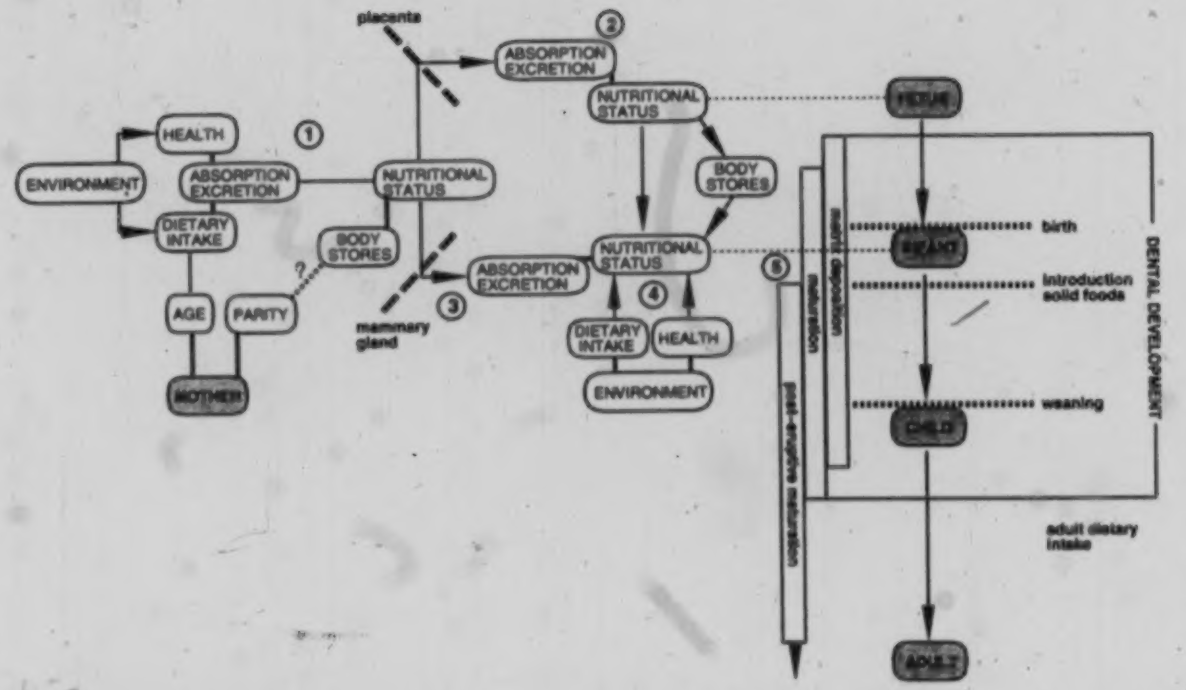

placenta
②
ABSORPTION EXCRETION
NUTRITIONAL STATUS
FETUS
HEALTH
①
ENVIRONMENT
ABSORPTION EXCRETION
NUTRITIONAL STATUS
BODY STORES
DIETARY INTAKE
BODY STORES
?
NUTRITIONAL STATUS
INFANT
birth
ABSORPTION EXCRETION
⑤
introduction solid foods
AGE
PARITY
mammary gland
③
④
DIETARY INTAKE
HEALTH
MOTHER
ENVIRONMENT
matrix deposition
maturation
post-eruptive maturation
weaning
CHILD
DENTAL DEVELOPMENT
adult dietary intake
ADULT

Fig. 3.2: Diagrammatic representation of trace element pathways between mother and fetus or infant, in relation to the three different stages of dental development (matrix deposition, maturation and post-eruptive maturation). The major components of the model (labels 1-5) are discussed in the text. This figure is an extended version of the model shown in Fig. 1.1 (Chapter 1).

Modelling of trace element pathways in relation to enamel composition

The information presented above has been used to construct a more elaborate model describing the trace element pathways into enamel (Figure 3.2). The discussion of the nutritional and medical literature shows that there are still many gaps in our understanding of trace element transfer between a mother and the developing fetus or infant. The fact that studies often report contradictory results emphasises the preliminary nature of the model presented here. The fact that there are so many variables involved, often beyond the control of the researcher, is a major problem in this area. Nevertheless, several factors that contribute to fetal and infant nutritional status, and thus to the circulating levels of trace elements, have been identified. In the following section, the findings for the selected elements will be discussed in relation to Figure 3.2. This discussion will reflect the difference in amount of available information for each of the elements.

Five main components comprise the model:

1) **Maternal nutritional status:** Maternal nutritional status is determined by maternal dietary intake, physiology and metabolism (absorption and excretion mechanisms, maintenance of body stores of trace elements), and factors such as her health and age. In addition to its direct effect on diet, the environment may indirectly affect absorption and excretion of elements through an effect on the health of the individual. The environment also plays a role in exposure to contaminants such as heavy metals (e.g., Pb).

2) **Placental transport and fetal nutritional status:** During pregnancy, trace elements and other metabolites are transferred from the maternal to the fetal blood circulation across the placental barrier. As we have seen in this chapter, there are both active and passive transport mechanisms, and the placenta can behave in a number of ways towards the trace elements: active (selective preference), passive (no specific effect), and discriminating (partial blocking of trace element transport).

Depending on the nutrient transfer during pregnancy, the fetus can accumulate significant body stores of Cu and Fe, and moderate stores of Zn. These trace elements are mobilized in the maternal body through adaptive responses such as increased retention or decreased excretion. Some elements, such as Pb, may be mobilized from maternal bone stores in association with changes in Ca metabolism. During fetal development, various systems for absorption and excretion are maturing (e.g., Mn metabolism).

3) **Mammary gland transport and infant nutritional status:** Various control mechanisms appear to regulate trace element secretion into milk. As for the placenta, the mammary gland can behave differently towards the different elements: active, passive, and discriminating. The concentrations of most of the elements in breast milk decline with ongoing lactation. Infant nutritional status during lactation is maintained by intake of nutrients via breast milk, as well as by body stores of some trace elements (Cu, Fe, Zn).

4) **Weaning and post-weaning diet:** During the early postnatal period, the infant's gastro-intestinal tract is maturing, which may change homeostatic control mechanisms for various trace elements. The gradual introduction of weaning foods exposes the infant to environmental influences via various solid foods and water. Complementary foods may affect absorption of trace elements from breast milk for the remainder of lactation, and may in fact alter the composition of the milk via indirect effects on suckling frequency and duration. Infant and child nutritional status may be affected by illness, which may occur more frequently once the passive immunity from the breast milk is no longer available.

5) **Dental development:** Three separate stages characterize dental development: matrix deposition, maturation, and post-eruptive maturation. These stages are not separated in time, however. In a developing tooth, both matrix deposition and maturation will be taking place simultaneously. Two of the crucial factors in determining the potential of trace element composition for palaeodietary studies are the timing of trace element uptake by the dental tissues, and the timing of the maturation process.

In addition to the filtering effects that take place in placenta and mammary gland, the ameloblast layer can present yet another barrier for the uptake of trace elements into the developing enamel.

Major elements: Calcium and phosphorus

Calcium and P are the main constituents of the hydroxyapatite component of bone and teeth. For these two elements, there is no (measurable?) effect of maternal dietary intake, either during the period *in utero*, or during lactation, on fetal or infant Ca levels. The transfer of both elements appears to be regulated. The finding of similarities in the changes in milk Ca level with lactation for women of contrasting socio-economic status shows that milk Ca levels are relatively independent of dietary adequacy.

The increased rate of transfer towards the end of gestation reflects the increased needs of the growing infant. Most of the Ca and P will be used in skeletal formation, and, unless there is a major disturbance in either maternal or fetal/infant Ca metabolism, the dental tissues will probably receive an adequate supply of Ca and P. Since Sr^{2+} and Ba^{2+} compete with Ca^{2+} at the level of cellular transport, high levels of these trace elements in the diet could theoretically result in reduced Ca levels in developing hard tissues.

Essential trace elements: Cu, Fe, Mn, Mo, V, and Zn

Copper

During pregnancy, Cu is mobilized in the mother to meet the needs of the infant. However, the element appears to be partially blocked during transfer across the placenta. This, and the fact that the fetus can excrete Cu via the umbilical artery in order to maintain homeostasis (Rossipal *et al.*, 2000), indicates that maternal dietary intake is not directly reflected in the amounts reaching the fetus.

Maternal Cu intake, whether supplemented or not, does not appear to affect the concentrations of this element in milk. Instead, the concentrations are individually regulated, with some mothers having higher, and some having lower Cu levels. Generally, Cu levels decline gradually with ongoing lactation, and Cu intake for breastfeeding infants is relatively low. The concentration of Cu in human milk or formula does not appear to have an effect on infant serum Cu levels, which suggests that Cu levels are maintained by the liver Cu stores, which are usually sufficient for the first 4-6 months after birth.

During gestation and breastfeeding, circulating Cu levels in the fetus and infant will not directly reflect maternal dietary intake. Generally, the fetus and suckling infant will probably obtain sufficient amounts of this element. However, once the liver Cu stores are exhausted, dietary intake will more directly affect Cu status. Infants with low Cu stores may develop Cu deficiency. Suboptimal dietary intake of Cu is reflected in suboptimal levels of Cu in the blood (Davis & Mertz, 1987). In contrast, substantially higher dietary levels of Cu are necessary to increase blood Cu levels. Thus, although normal or relatively high Cu intake may not be reflected in developing tissues, a low dietary Cu intake may result in low Cu in teeth.

In enamel, Cu is probably mostly associated with the organic phase. Thus, during formation, most of the copper ions will be taken up during the matrix formation stage, and subsequently be lost during maturation. The concentration of Cu in enamel is thus expected to not directly reflect the levels of Cu in the diet at the time of formation. In addition, Cu levels have been found to be variable among women: differences in enamel Cu concentrations among individuals need not necessarily reflect differences in dietary intake, but could derive from other, e.g. genetic, factors.

Iron

During pregnancy, Fe is mobilized from maternal stores. The rate of transport to the fetus increases towards the end of gestation. All the Fe transported across the placenta is retained by the fetus, and relatively large amounts are stored in fetal haemoglobin. The fact that maternal stores are mobilized suggests that there is no direct relation between maternal dietary intake during pregnancy and fetal Fe levels. If maternal dietary intake has been inadequate for some time, her Fe stores might be insufficient to meet the needs of the growing fetus as well as her own needs. It appears, however, that when mother and fetus compete for limited Fe, the mother is more likely to develop anaemia than the fetus or infant, suggesting some kind of regulation (protection?) of the fetal Fe supply.

The concentration of Fe in breast milk shows a gradual decline with ongoing lactation. There is no conclusive evidence to show that maternal dietary intake, whether supplemented or not, affects milk Fe levels. Generally, Fe intakes are relatively low for breast fed infants. However, under normal circumstances the Fe stores, which were accumulated during gestation, will be sufficient to meet the infant's needs for the first few months after birth. As with Cu, infants with low body stores of Fe (preterm or low birth weight infants) may develop Fe deficiency around 2-3 months after birth.

For the dental tissues developing *in utero*, Fe levels are not expected to directly reflect maternal dietary intake. Instead, Fe transport across the placenta will be a function of overall maternal Fe status, which is a composite of maternal dietary intake and health status prior to, and at the time of, pregnancy. Because the Fe requirements of the fetus appear to be met, to some extent, at the expense of maternal needs, one would expect that most fetuses have a reasonably adequate Fe supply. Although fetal stores may be either smaller or larger at birth, it is possible that circulating levels of Fe are comparable for most fetuses. The developing teeth are therefore not necessarily very different in their Fe concentrations. After birth, once the Fe stores have been used and dietary intake remains low, or Fe bioavailability is reduced due to the introduction of, for example, cereal foods, infants may develop Fe deficiency anaemia. It is conceivable that this condition is reflected in low Fe concentrations in enamel.

Iron appears not to be incorporated during maturation. Most of the Fe in enamel, therefore, will be either built into the enamel during matrix deposition, or taken up into surface layers of enamel after eruption. Based on the above, the Fe levels in deciduous teeth, and in part of the first permanent molar crown, will likely not reflect maternal dietary intake of this element. Possibly, inadequate levels of Fe intake are reflected in their concentrations in enamel. However, Fe from post-eruptive uptake may diffuse into deeper enamel layers, obscuring the original signal.

Manganese

Manganese is actively transported across the placenta and levels of this element in fetal serum are substantially higher than those in the mother. Because Mn levels in milk may reflect maternal dietary intake of this element, it is conceivable that Mn levels transported across the placenta also show a correlation with maternal intake, although this has not been reported in the literature discussed in this chapter. During the early stages of fetal development, manganese metabolism is still developing and the element is not stored - as far as is known – so that circulating Mn levels are not yet fully adjusted by various homeostatic mechanisms. This may mean that Mn concentrations in the fetal tissues may in fact directly reflect Mn 'intake'.

As mentioned above, Mn in human milk may reflect maternal dietary intake. For breast fed infants, serum Mn shows a correlation with Mn intake from breast milk. Dental enamel formed during the breastfeeding period, therefore, may indeed correspond with levels of this element in the diet. As discussed in Chapter 2, Mn levels in teeth have been found to increase with increasing levels of this element in the diet. However, if Mn is associated with the organic phase, as suggested by Ung Bao (1990), most of the Mn may be removed from the enamel during maturation, thereby disturbing the original dietary signal.

A substantial drop in serum Mn values (ca. 50%), from their initial high values to the lower adult levels, begins around 6 months of life, continuing on until about 18 years. The onset of this change partly corresponds to the maturation of Mn metabolism, and can coincide with various changes in trace element intake associated with the introduction of complementary foods, which will often occur around 6 months of age as well. Thus, although Mn may be a dietary indicator, showing relatively higher levels in enamel formed during the first few months of life, several factors may complicate the interpretation of Mn concentrations of enamel samples.

Molybdenum

The placenta does not appear to have any specific effect on the transport of this element to the fetus. Thus, if maternal dietary intake and serum levels are related (not known?), it is possible that fetal serum levels reflect maternal dietary intake. However, Mo can be excreted by the fetus via the umbilical cord, which indicates that a degree of homeostatic control is already present at this time.

The concentrations of Mo in human milk gradually decrease with ongoing lactation, as with a number of other elements. There may be regional variation in milk levels of this element, reflecting local environmental circumstances (possible association with Mo levels in the soil).

Levels of Mo in enamel are generally very low. The element was not found in unerupted and newly erupted teeth (Table 2.6), which suggests uptake of this element is exclusively post-eruptive. This does not, however, agree with reports of Mo uptake during the maturation stage (see Chapter 2) and with the fact that concentrations of this element have been found to increase with increasing dietary levels. A possible explanation for this apparent contradiction is that the concentrations are so low that the element is not always detected, depending on the analytical technique used.

Vanadium

Vanadium can cross both the placenta and mammary gland. However, the placenta itself appears to retain most of this element, so that there is no direct correlation between serum levels in the fetus and mother. Levels in milk may reflect local environmental circumstances. Vanadium appears to be preferentially incorporated into bone, and, by inference, in enamel. Given the scarcity of information on this element in the literature it is not possible at this time to draw any conclusions about the potential of V as a (palaeo)dietary indicator.

Zinc

Zinc, like Cu and Fe, is mobilized in the mother during pregnancy. Therefore, it is not expected that Zn transport to the fetus directly reflects maternal dietary Zn intake at the time of pregnancy. The fetus accumulates moderate stores of the element during development, which are then used during the first few months after birth. The level of Zn in breast milk shows a continuous decrease, which is quite steep during the first few months and then becomes more gradual with ongoing lactation. There is no conclusive evidence for a straightforward relation between maternal dietary intake and milk Zn levels. For the breastfeeding infant, intake of Zn from milk is, at least during the first few months after birth, complemented by internal redistribution of Zn from liver stores. During this period therefore, serum Zn levels, representing Zn available for incorporation into developing teeth, do not necessarily correspond with dietary intake. In fact, serum Zn levels appear to decrease during this period. Once the liver Zn stores have been consumed, maintenance of adequate Zn levels largely depends on dietary intake, and there is a risk of Zn deficiency. Thus, it is possible that Zn concentrations in enamel formed after the first few months of life (*i.e.*, especially the enamel in permanent teeth) do correspond to dietary Zn levels. The major deposition of Zn in enamel takes place before eruption. The concentration of Zn has been reported to increase with increasing dietary intake.

Non-essential trace elements: Ba, Sr and Pb

Barium and strontium

These two elements are chemically very similar and tend to follow Ca in metabolic pathways. As was mentioned above, Ba^{2+}- and Sr^{2+}-ions can compete with Ca^{2+} during placental transport and, probably, during transport across the mammary gland. However, there is a physiological discrimination against Ba and Sr in favour of Ca. This discrimination may decrease towards the end of pregnancy.

There is no conclusive evidence with regard to the level of Ba in breast milk. It may show a gradual decrease with ongoing lactation, and it has been suggested that Ba levels in milk are responsive to maternal dietary intake. Barium concentrations in teeth were found to reflect concentrations of Ba in the diet (Healy & Ludwig, 1968).

Strontium concentrations in milk do not show any consistent trend with ongoing lactation. Strontium levels in teeth show geographic differences that have been ascribed to variation in Sr concentrations in food and (especially) drinking water (Steadman *et al.*, 1958; Gedalia, 1975; Little & Barrett, 1976).

Both trace elements take up Ca positions in the hydroxyapatite crystals. Strontium appears to be mainly taken up during the maturation stage, and is not generally taken up post-eruptively.

Lead

This heavy metal appears to cross the placenta readily, as well as the mammary gland. Levels of Pb transported to the fetus or the infant reflect maternal blood levels, which in turn reflect environmental exposure to this element. This can also include exposure to Pb prior to pregnancy, because Pb is remodelled from the maternal skeleton in association with changing Ca metabolism. Lead is incorporated into enamel mainly post-eruptively, and shows a steep gradient from the surface of enamel inward. The Pb profile of teeth is particular to the individual and Pb levels correspond to environmental levels. Within a dentition, different teeth tend to show different concentrations of enamel according to the time of eruption (*i.e.*, total post-eruptive time).

Summary

For palaeodietary reconstructions we are interested in relative levels of intake of certain food types as well as the timing of changes in dietary intake, such as weaning.

Palaeodietary methods are based on the use of certain trace elements as markers for certain food categories (see Appendix B). In enamel, levels of Ba, Mn, Mo, and Zn have been found to be related to levels of these elements in the diet. Strontium levels appear to mainly reflect the levels in drinking water, whereas Pb concentrations correlate with amounts of this element in the environment.

The incremental structure of enamel can – in theory – provide us with a chronological framework for the compositional information. For reconstructions of weaning patterns in prehistoric populations, we would need to know the timing of the introduction of complementary foods, and the timing of complete cessation of breastfeeding (markers 2 and 3 in Figure 3.1). Breastfeeding patterns have been demonstrated in archaeological populations based on stable isotope analysis of dental enamel samples. The information presented in this chapter, with regard to trace element composition of human milk, suggests that it is much more difficult to track changes in feeding patterns over time on the basis of the trace element composition of teeth.

A number of trace elements in human milk (Cu, Fe, Mo, Zn, and possibly Ba) show a gradual decrease in concentration with ongoing lactation, so that the composition of breast milk (*i.e.* the infant's dietary intake) over time is not constant. The introduction of complementary foods (marker 2) can affect the composition of human milk indirectly through the resulting changes in frequency or duration of breastfeeding. In addition, the non-milk foods can directly affect the availability of trace elements from milk. For example, phytates can affect the bioavailability of Zn and Mn.

The age at which complementary foods are introduced is variable, but usually this is around 4-6 months. It is often recommended to start feeding the infant other foods around this age because body stores of Cu, Zn and especially Fe, which were created *in utero*, will become depleted at this age. Thus, around this time breast milk should be supplemented in order to prevent deficiencies of these elements. If dietary intake of these elements is insufficient, it may be possible to detect deficiencies of these elements in enamel developing at that time.

Around the time of the introduction of complementary foods, a number of changes will take place simultaneously: depletion of body stores, changes in breast milk composition, and changes in the bioavailability of several trace elements from human milk because of other food components. It will be very hard to predict any shifts in trace element composition of enamel that will be indicative of this stage (marker 2). Several studies have focused on the use of Sr/Ca and Ba/Ca ratios in enamel samples for this purpose (Ehlken, 1991; Grupe & Bach, 1993; Siegert, 1993; see also Grupe, 1998).

A marker for stage 3 (complete cessation of breastfeeding) seems even more elusive. The relative contribution of breast milk to total dietary intake of the infant will be very small. There does not appear to be a trace element that is highly specific to breast milk, and could be used as a marker (cf. the isotopic enrichment on which stable isotope methods of determining weaning age are based).

Based on the information presented in this chapter, it seems particularly difficult to determine dietary intake/adequacy from deciduous tooth samples. Various factors ensure that the fetus can develop in a very protected environment, which generally means that adequate amounts of trace elements will be available to the developing tissues. During the first 4-6 months after birth (when the majority of deciduous tooth enamel formation takes place), the internal redistribution of body stores of certain elements, as well as the maturation of various metabolic and physiological processes complicate relations between dietary intake and enamel composition. It is possible that for infants who are born with low body stores (for example premature or low birth weight infants) certain deficiencies could be detected. However, it is questionable that such infants would have survived in prehistoric times.

Of the permanent teeth, first molars, and possibly permanent central incisors, will be developing when complementary foods are being introduced. The remainder of the teeth develop during a period when the individual's metabolism and homeostatic mechanisms will have fully matured. Some trace elements have the potential to serve as indicators of dietary intake or adequacy. To what extent we will be able to use the incremental structure to determine more accurately at what age certain dietary 'events' took place, depends entirely on our understanding of the mode and timing of the enamel maturation processes. As was discussed in Chapter 2, there are still significant gaps in our knowledge of enamel maturation and especially the timing and duration of the separate stages and phases. Although the incremental structure allows us to determine aspects of crown formation in relation to developmental time, it is not necessarily true that the composition of enamel can be linked directly to that information.

CHAPTER 4

MATERIAL AND METHODS

Description of samples

Permanent Teeth

One maxilla of a subadult, containing 11 teeth (Fig. 4.1) was obtained from a collection of skeletal remains of unknown provenance housed in the Department of Anatomy, University of Groningen (The Netherlands). This collection derived from archaeological excavations carried out by the Biological Archaeological Institute (now the Groningen Institute of Archaeology) early in the 20th century. The maxilla was selected because the 11 teeth were all relatively unworn and free of caries. Based on the stage of development of the third molar roots this individual was ca. 15 years old. Although no further information is available about this specimen other than that it is probably (and at most) a few hundred years old, it is well suited for the purposes of the current investigation into compositional variation of enamel within a single dentition, and permission could be obtained to apply destructive analytical techniques. Samples were labelled: P-LC (left canine), P-LP3 (left first premolar), P-LP4 (left second premolar), P-LM1 (left first molar), P-LM2 (left second molar), P-LM3 (left third molar) and P-RP3, P-RP4, P-RM1, P-RM2, P-RM3 for the corresponding right teeth.

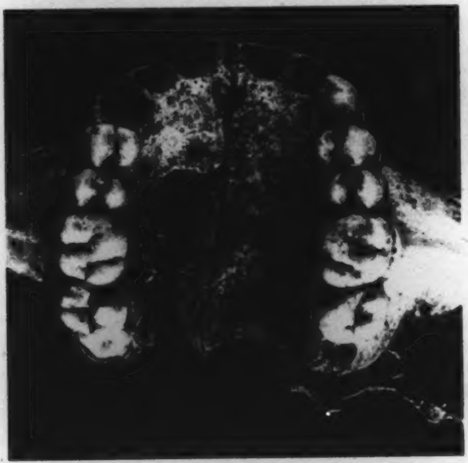
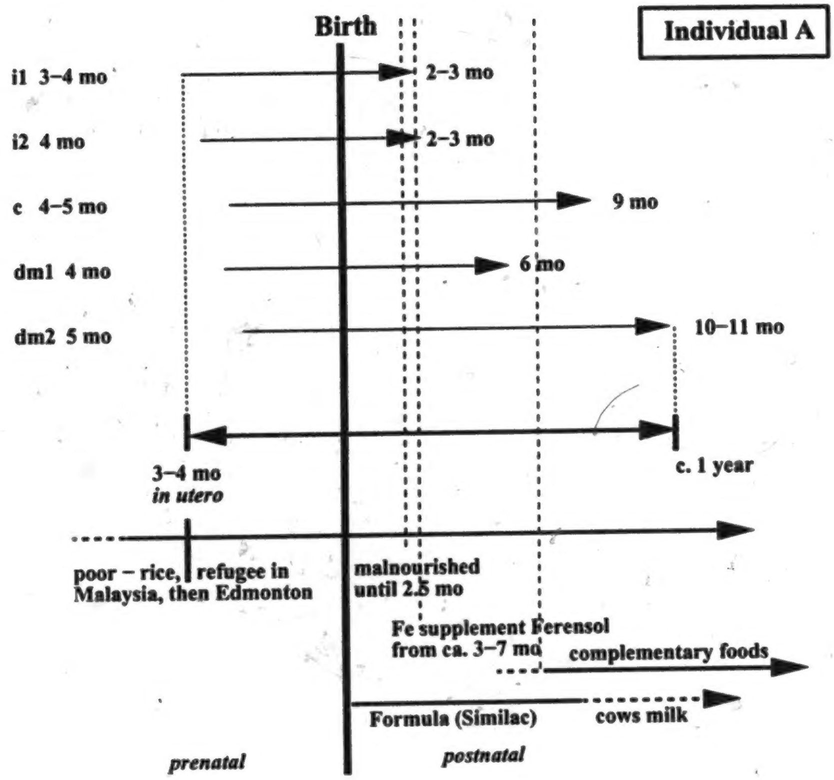


Fig. 4.1: Maxillary permanent teeth of a subadult from an archaeological excavation in the Netherlands.

Table 4.1: Overview of all the specimens used in this study, with the labels used throughout the text, and a description specifying the tooth type, location within the jaw (if known), and origin of specimen. All permanent tooth labels begin with a 'P'; the labels for all the test samples begin with a 'T'; the labels for the deciduous teeth all begin with the letter used to designate the individual.

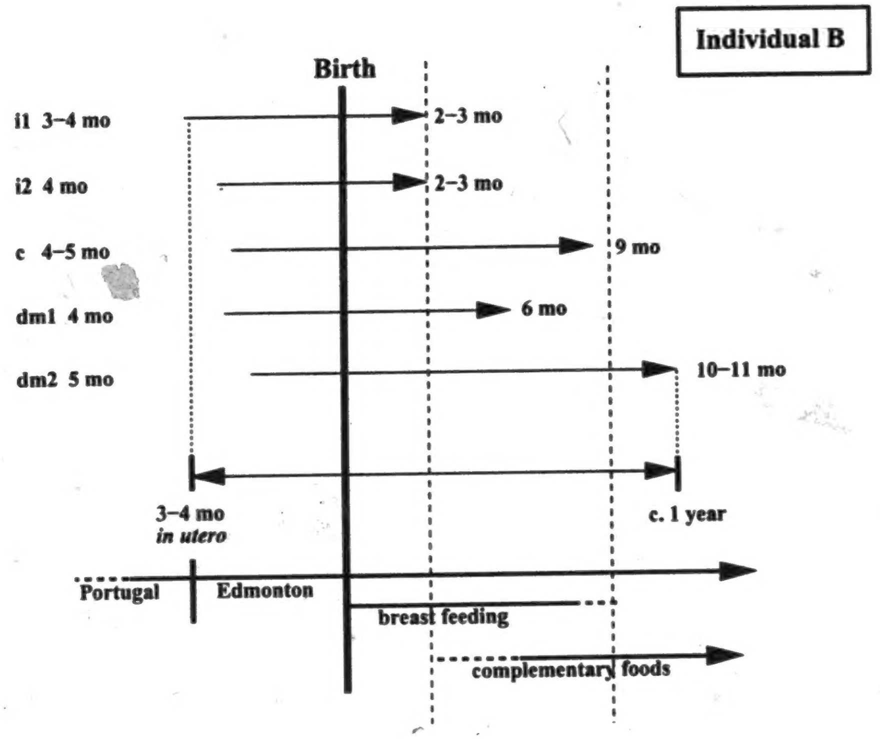
Subset	Label	Description
Permanent teeth	P-LC	Maxillary left canine, archaeological specimen (the Netherlands)
	P-LP3	Maxillary left first premolar, archaeological specimen (the Netherlands)
	P-LP4	Maxillary left second premolar, archaeological specimen (the Netherlands)
	P-LM1	Maxillary left first molar, archaeological specimen (the Netherlands)
	P-LM2	Maxillary left second molar, archaeological specimen (the Netherlands)
	P-LM3	Maxillary left third molar, archaeological specimen (the Netherlands)
	P-RP3	Maxillary right first premolar, archaeological specimen (the Netherlands)
	P-RP4	Maxillary right second premolar, archaeological specimen (the Netherlands)
	P-RM1	Maxillary right first molar, archaeological specimen (the Netherlands)
	P-RM2	Maxillary right second molar, archaeological specimen (the Netherlands)
	P-RM3	Maxillary right third molar, archaeological specimen (the Netherlands)
Test samples NAA	T-C_1	Modern canine, upper portion (sectioned as per Fig. 4.9 B)
	T-C_2	Modern canine, middle portion (sectioned as per Fig. 4.9 B)
	T-M3-1	Modern third molar, middle section (sectioned as per Fig. 4.9 A)
	T-M3-2	Modern third molar, middle section (sectioned as per Fig. 4.9 A)
	T-M3-3	Modern third molar, outer section (sectioned as per Fig. 4.9 A)
	T-M3-4	Modern third molar, outer section (sectioned as per Fig. 4.9 A)
	T-3A-3	Fragment from a prehistoric specimen from Algeria, ca. 8000 years old
	T-Dist. Orig.	Fragment from an isolated archaeological tooth from the Netherlands
	Modern incisor (T)	Modern tooth used for microprobe analysis (results reported Fig. 5.3)
Deciduous teeth	A-Li1	Maxillary left central incisor, individual A
	A-Ri2	Maxillary right lateral incisor, individual A
	A-Rm1	Maxillary right first molar, individual A
	A-Rm2	Maxillary right second molar, individual A
	B-Ri1	Maxillary right central incisor, individual B
	B-Ri2	Maxillary right lateral incisor, individual B
	B-Rc	Maxillary right canine, individual B
	B-Rm1	Maxillary right first molar, individual B
	B-Rm2	Mandibular right second molar, individual B
	C-Ri1	Maxillary right central incisor, individual C
	C-Lc	Mandibular left canine, individual C
	C-Lm1	Maxillary left first molar, individual C
	C-Lm2	Maxillary left second molar, individual C
	D-L?c	Mandibular left (?) canine, individual D
	E-Li1	Maxillary left central incisor, individual E
	F-Li2	Mandibular left lateral incisor, individual F
	F-Lm2	Mandibular left second molar, individual F


Birth
Individual A
i1 3–4 mo
2–3 mo
i2 4 mo
2–3 mo
c 4–5 mo
9 mo
dm1 4 mo
6 mo
dm2 5 mo
10–11 mo
3–4 mo
in utero
c. 1 year
poor – rice, refugee in Malaysia, then Edmonton
malnourished until 2.5 mo
Fe supplement Ferensol from ca. 3–7 mo
complementary foods
Formula (Similac)
cows milk
prenatal
postnatal

Prenatal diet
Poor – rice. Mother refugee in Malaysia, then in Edmonton.

Postnatal diet
- **Malnourished until entered hospital – weight 4300 g – at 2.5 mo**
- **Left hospital at 3 mo. Low level of haemoglobin so Fe supplement Ferensol until ca. 7 mo when Hb = 12 +.**
- **Similac (milk formula) supplemented with solids from ca. 6–7 mo.**
- **Cows milk from ca. 9 mo; lots of milk and cheese after that.**

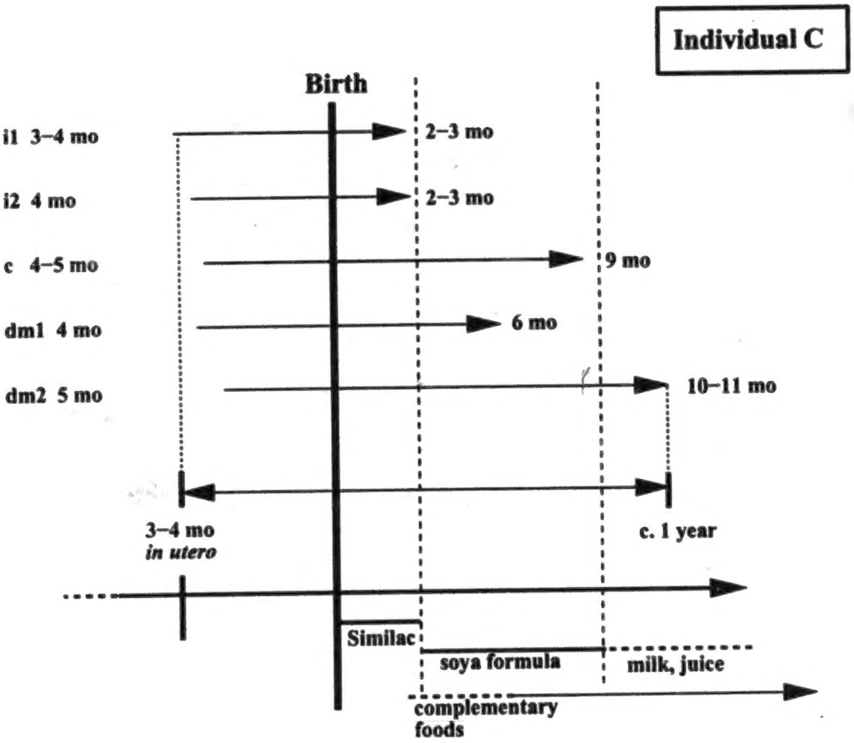
Fig. 4.2: Diagram showing generalized dental development (based on Table 2.2) and dietary records for individual A.


Individual B
Birth
i1 3–4 mo
2–3 mo
i2 4 mo
2–3 mo
c 4–5 mo
9 mo
dm1 4 mo
6 mo
dm2 5 mo
10–11 mo
3–4 mo
in utero
c. 1 year
Portugal
Edmonton
breast feeding
complementary foods

Prenatal diet
Portugal to 4th month (marine fish and shellfish, barbecued chicken, tomatoes, potatoes, olive and vegetable oils) then Edmonton.

Postnatal diet
- **Breast fed until around 9 months (maternal diet: Ca from non–dairy sources – possibility of Pb from Ca supplements)**
- **Supplemented from around 3–6 months – general diet: milk, peas, potatoes, fruit, fish, wheat, etc.**

Fig. 4.3: Diagram showing generalized dental development (based on Table 2.2) and dietary records for individual B.


Individual C
Birth
i1 3–4 mo
2–3 mo
i2 4 mo
2–3 mo
c 4–5 mo
9 mo
dm1 4 mo
6 mo
dm2 5 mo
10–11 mo
3–4 mo in utero
c. 1 year
Similac
soya formula
milk, juice
complementary foods

Prenatal diet
Very poor – rice. In Edmonton throughout.

Postnatal diet
- Similac until ca. 4 mo
- Prosobee (soya formula) until ca. 10 mo; then milk or juice.
- Supplemental foods (solids) from 3–6 mo.
- Some milk but lactose intolerant (diarrhoea until age 2 y).

Fig. 4.4: Diagram showing generalized dental development (based on Table 2.2) and dietary records for individual C.

Deciduous Teeth

Deciduous teeth from six individuals were available for study. For three of these individuals (A (female), B (female) and C (male)) the dentitions were almost complete. In addition, records containing information about health, general aspects of diet and dates of eruption and shedding of these teeth were available (Figures 4.2 – 4.4; Tables 4.2 – 4.4), which makes this a unique sample.

Table 4.2: Eruption and shedding of the deciduous teeth of individual A. R=right; L=left; E=extracted. For abbreviations used for the teeth (di1, etc.) See List of Abbreviations included in the prefatory pages.

tooth	Eruption (age in days)	Eruption (age in months)	Shed (age in years)
Lower di1R	177	5.9	5.7
Lower di1L	179	5.9	5.9
Upper di1L	192	6.4	6.4
Upper di1R	200	6.7	6.3
Upper di2L	211	7.0	7.4
Upper di2R	233	7.8	7.4
Lower di2L	247	8.2	6.7
Lower di2R	342	11.4	E 6.8
Upper dm1R	362	12	8.9
Upper dm1L	376	12.5	?
Lower dm1L	417	13.9	?
Upper dcR	438	14.6	9.2
Upper dcL	441	14.7	9.3
Lower dm1R	453	15.1	8.8
Lower dcL	482	16.1	E 6.9
Lower dcR	502	16.7	E 6.8
Lower dm2L	738	24.6	11 (broke in half)
Lower dm2R	744	24.8	8
Upper dm2L	782	26.1	11.3
Upper dm2R	1025	34.2	10.2

Table 4.3: Eruption and shedding of the deciduous teeth of individual B. R=right; L=left. For abbreviations used for the teeth (di1, etc.) See List of Abbreviations.

tooth	Eruption (age in days)	Eruption (age in months)	Shed (age in years)
Lower di1R	219	7.3	6.1
Lower di1L	223	7.4	6
Upper di1R	319	10.6	7
Upper di1L	334	11.1	7.2
Upper di2R	368	12.3	7.4
Upper di2L	437	14.6	7.9
Lower di2L	442	14.7	6.8
Upper dm1R	458	15.3	10.3
Upper dm1L	463	15.4	10.3
Lower di2R	481	16.0	6.8
Lower dm1R	491	16.4	10.5
Lower dm1L	497	16.6	10.8
Upper dcR	572	19.1	10.2
Upper dcL	572	19.1	10.3
Lower dcR	593	19.8	9.3
Lower dcL	595	19.8	9.4
Upper dm2R	715	23.8	10.4
Upper dm2L	715	23.8	10.3
Lower dm2L	738	24.6	10.9
Lower dm2R	779	26	11.2

Table 4.4: Eruption and shedding of the deciduous teeth of individual C. R=right; L=left; E=extracted. For abbreviations used for the teeth (di1, etc.) See List of Abbreviations.

tooth	Eruption (age in days)	Eruption (age in months)	Shed (age in years)
Lower di1L	288	9.6	6.5
Lower di1R	295	9.8	6.5
Upper di1L	354	11.8	E 7.2
Upper di1R	358	11.9	7.3
Upper di2R	417	13.9	8.9
Upper di2L	433	14.4	8.8
Upper dm1R	500	16.7	12.3
Upper dm1L	500	16.7	12.6
Lower di2L	502	16.7	E 6.6
Lower di2R	506	16.9	E 6.6
Upper dcL	551	18.4	?
Upper dcR	575	19.2	?
Lower dm1L	589	19.6	E 7.9
Lower dm1R	620	20.7	E 7.9
Lower dcL	623	20.8	E 6.6
Lower dcR	631	21	E 6.6
Lower dm2R	1049	35	E ?
Lower dm2L	1079	36	E ?
Upper dm2R	1110	37	13.1
Upper dm2L	1192	39.7	13.6

Additional specimens were obtained from three other individuals. For the two siblings D and E (both males) some information was available about the feeding regime during the first year of life (including breast/bottle feeding and weaning; Table 4.5). These two individuals were born in different places in Canada (Hamilton, Ontario, and Edmonton, Alberta, respectively). Unfortunately, many of their deciduous teeth were not suitable for the purposes of this study because of the presence of fillings. For the sixth individual (F - female), several teeth were available, but no records about dietary intake.

Table 4.5: Information available for individuals D and E.

ID	Birth	Breastfed until	Weaning diet
D	1 month premature Hamilton	5 months	Apple juice, formula, rice, banana, carrots, chicken, peas, biscuits, cheese; Full adult diet by 18-24 months
E	2 weeks premature Edmonton	8 months	Milk, juices; see above Full adult diet by 18-24 months

Expectations regarding trace element composition of enamel for the individuals with records

Expectations based on the palaeodietary method

Palaeodietary reconstructions are based on the knowledge that certain trace elements occur predominantly in association with certain types of foods. Appendix B includes a table listing selected food sources for each of the selected trace elements. Using this information, a set of fairly general expectations can be put forward with regard to expected trace element concentrations, or the timing of changes in trace element concentrations, in the enamel samples from the individuals with dietary records.

In the case of individual A (Fig. 4.2), maternal diet was poor and rice-based. The mother moved between different countries during the 4th or 5th month of pregnancy. Movement was from an area in which fresh marine resources were available, to one in which such resources were absent. The concentrations of several elements vary geographically, such as Sr in drinking water and Mo in soil, and this may have resulted in a change in maternal dietary intake of these elements. We may assume that the maternal diet, described as 'poor', involved mostly vegetable foods and little or no meat, corresponding to relatively high Sr, Mn, possibly V, and low Cu and Zn. It is even possible that the maternal diet was deficient in a number of nutrients, although we have no way of knowing which. There was, however, prenatal care in later pregnancy. Individual A was malnourished during the early post-natal period and received Fe-supplements to treat a low level of haemoglobin. Thus, the period from about 3-7 months after birth is characterized by comparatively higher Fe-intake than before. This individual received milk formula, supplemented with solid foods from about 6-7 months. The introduction of solids is often characterized by an increase in dietary Sr/Ca ratio.

For individual B (Fig. 4.3), the prenatal maternal diet included various marine resources (fish, shellfish) and meat for the first 4 months, corresponding to high levels of Cu, Sr and Zn. Barium was found to be low in marine sources (Wessen *et al.*, 1977), but there appears to be a wide variation in this element in both plant and animal foods (Nielsen, 1986). At 4 months *in utero* there was a geographic relocation, which may have involved a change in Sr and/or Mo levels, as was the case for individual A. Maternal diet also included non-dairy Ca sources and Ca-supplements, which may have been contaminated with Pb. Supplemental foods were introduced around 3-6 months. The shift in Sr/Ca ratio is therefore expected slightly earlier than for A, but breastfeeding was continued until about 9 months.

For individual C (Fig. 4.4), as for A, maternal diet was poor and based on rice. We may similarly postulate low Cu and Zn (no meat) and high Sr, Mn, and possibly V, for vegetable-based foods. There was no prenatal care, *i.e.* no dietary monitoring or supplementation. This individual's mother lived in the same geographic area (one with no fresh marine resources) throughout pregnancy, so that general environmental levels, and thus dietary intake, of Sr and Mo were likely the same throughout. This individual received formula; initially milk-based (Similac) but at ca. 4 months of age this was replaced by a soy-based formula (Prosobee), which was continued until ca. 10 months of age. As for individual B, solids were introduced from around 3-6 months.

Table 4.6: Summary of the expectations based on the palaeodietary method.

[illegible]	[illegible]	[illegible]
High: Sr, Mn, Fe, (V) Low: Cu, Zn Changes: Sr, Mo Sr/Ca ratio change from 6-7 months	High: Cu, Sr, Zn (Pb?) Changes: Sr, Mo Sr/Ca ratio change from 3-6 months	High: Sr, Mn, (V) Low: Cu, Zn No changes: Sr, Mo Sr/Ca ratio change from 3-6 months

In summary, dental enamel samples from the three individuals are expected to differ in the following way (see also Table 4.6):

A, C vs. B: levels of Cu, Zn
A, B vs. C: levels of Sr, Mo
A vs. B, C: timing of change in Sr/Ca ratio

Expectations based on the model

The model presented in Chapter 3 allows for a slightly more detailed set of expectations, using other sources of information in addition to the dietary records used above. The model allows us to take into consideration the following aspects:

1. The relation between maternal dietary intake and transport of trace elements across placenta and mammary gland; knowledge of milk composition and differences between human milk and different types of infant formula;
2. Additional information (not usually available for archaeological material)
    a. Health records
    b. Premature/term infants.

It should be emphasised however, that the model as presented here is only preliminary. It still has many gaps, and the studies on which it is based involve many uncertainties and contradictions.

In order to generate a second set of expectations, the five individuals for whom dietary records are available can be subdivided in several ways: geographic location, formula (cow's milk based or soy milk) or breast milk, and term or premature. Each of these classifications leads to certain predictions in terms of expected trace element levels in enamel (Table 4.7). As we have seen in Chapter 3, various mechanisms play a role in the transfer of trace elements from mother to fetus or infant. Therefore, the predictions have been adjusted by taking into account these factors.

Four of the five individuals were born in Edmonton (Alberta, Canada), whereas one individual (D) was born and raised in Hamilton (Ontario, Canada). This individual may therefore have had a different intake of Sr (from drinking water) compared to the other individuals. In addition, Mo and V concentrations in breast milk may vary geographically.

A subdivision into formula-fed and breastfed leads to the following predictions: generally, the availability of Cu, Mn, and Zn is higher from human milk in comparison with formula. However, levels of Cu in milk appear to vary among women and this will complicate the picture. In addition, the model predicts that levels of Cu in enamel will not directly relate to dietary intake. Because of the high concentrations of Mn in soy formula, Mn absorption may actually be higher in formula fed infants than in breastfed infants. Zinc absorption is particularly low in infants fed soy formula. Serum Mo levels have been found to be higher in formula fed infants than breastfed infants. Both Mo and V may show regional variation in breast milk in relation to environmental levels. Levels of Ba, Sr and Pb appear to be higher in formula compared to breast milk.

Two individuals (D and E) were born prematurely, which may have resulted in slightly lower body stores of Fe, Cu and Zn. Individual C, being lactose intolerant, received little milk and had diarrhoea until age 2 years. This may have interfered with the uptake of various trace elements in the gastrointestinal tract.

The records of dietary intake are analyzed in the same way as is done using the palaeodietary method. However, the model predicts only relationships between maternal dietary intake and milk composition for Mn, Ba, and possibly Mo and V (as related to environmental levels). Copper, Fe and Zn are mobilized in the mother and transfer of these elements to the fetus does not directly reflect maternal dietary intake. However, since maternal diet was poor for individuals A and C, we could postulate that maternal body stores of Fe were low, and transfer of Fe and, possibly, Cu and Zn, to the fetus may have been suboptimal. For individual B maternal diet also included Ca from non-dairy sources, and the Ca supplements may have been contaminated with Pb.

In addition to the above mentioned factors that can be expected to alter the elemental composition of enamel, the model also contains numerous homeostatic processes that would dampen the effect of external (e.g., dietary) influences on enamel composition.

Table 4.7: Summary of the expectations based on various records and the model presented in Chapter 3.

	[illegible]		Breastfed		
	A	AC	B	E	D
	[illegible]	[illegible]	Until 9 [illegible]	Until 8 [illegible]	Until 5 [illegible]
Geography					Sr different (drinking water) Mo and V possibly different
[illegible] Breastfeeding		Mn possibly higher than all others; Zn low	(Cu?) Mn, Zn higher		
	Ba, Sr, Pb, Mo higher				
Birth: term or preterm	Term	Term	Term	Preterm: Lower body stores of Cu, Zn, Fe	
Health	Low Fe → Fe-supplements → Higher Fe	Diarrhoea until age 2 years: impaired absorption various trace elements			

Sample preparation

The samples to be used in the final analyses (*i.e.*, the deciduous teeth and the maxillary permanent teeth) were crucial to this study and could not easily be replaced. Therefore, it was essential to decide upon the proper sample preparation technique *prior to* analysis of these samples. Experimental work was carried out to test various methods of sample preparation. Those experiments were performed using modern teeth obtained from the Oral Surgery Clinic (University of Alberta).

Embedding or not?

It is technically possible to section teeth without embedding them in plastic. However, during one of the first trial projects an archaeological tooth was included in the analysis. This sample, from Casa da Moura (Portugal) was 5-6,000 years old and a scanning microscopy study showed that such samples are very friable (Fig. 4.5) and absolutely require embedding prior to sectioning. To generalize sample preparation methods for a wide range of samples (including friable archaeological specimens, as well as the smaller, much more difficult to handle deciduous teeth), and in order to facilitate thin sectioning for potential future histological analysis, it was decided that all specimens would be embedded in epoxy.

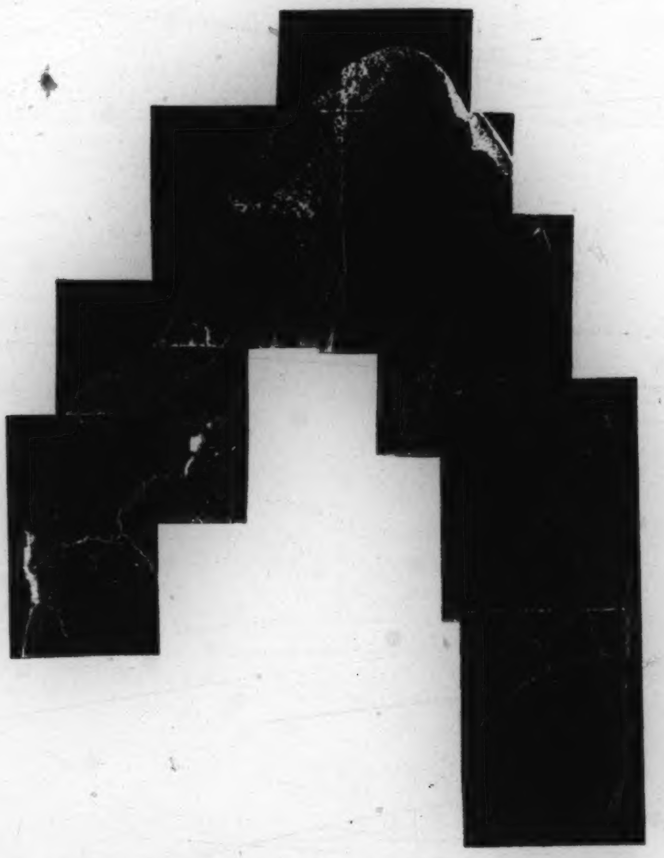


Fig. 4.5: SEM composite of a sectioned archaeological tooth from Casa da Moura, Portugal, ca. 5-6,000 years old, showing extensive cracking.

Separation of enamel and dentine

Traditionally, there are two categories of methods to separate enamel from dentine (Jenkins, 1978). The first consists of mechanical methods, an example of which would be to grind off all the dentine with a bur until only the enamel is left. This technique does not yield a 100% pure enamel sample, and there is a risk of losing material during the process.

The other category consists of flotation methods. Here the whole tooth is powdered, poured into a fluid with a density of 2.70, and then centrifuged. Because enamel has a density of 2.9-3.0 it will sink, while dentine (density 2.14) and cementum (density 2.03) will float. By repeating the procedure, the purity of enamel can reach about 97-99%. Although this method seems preferable to the mechanical methods, the fluids that are used, a mixture of 91% bromoform and 9% acetone or an aqueous solution of cadmium tungstoborate (82% w/w), may have an effect on the chemical composition of the sample. Therefore, the mechanical separation methods were deemed preferable since it does not involve the risk of contamination of the sample and/or alteration of the composition due to loss of components.

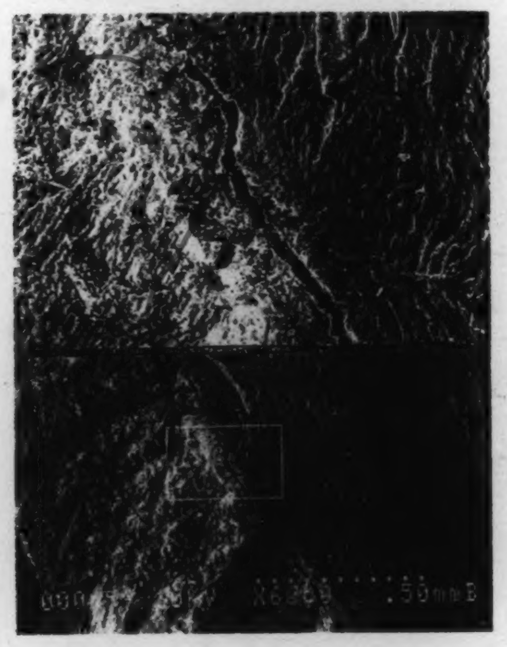


Fig. 4.6: Scanning electron micrograph showing cracking along the EDJ on a freeze-fractured incisor. Inset shows details. Left = enamel, right = dentine.

From SEM-images of archaeological specimens, as well as from air-dried modern specimens, it was known that fractures often occur in the vicinity of the enamel-dentine junction or EDJ (Fig. 4.6). Such fracturing is possibly due to the differential response of enamel and dentine to desiccation. Another possibility (for archaeological specimens) is that such fractures arise when specimens are subjected to freeze-thaw cycles.

In an attempt to split the enamel off the dentine along the EDJ, it was decided to mimic those long-term processes of freeze-thaw cycles or desiccation. In a series of experiments teeth were subjected to a 'thermal shock', induced by alternating rounds of high temperatures (hot plate) and very cold temperatures (liquid nitrogen). It was found that, although the enamel cap would sometimes come off the dentine, this was unpredictable and had a low rate of reproducibility. Sometimes the enamel would split according to previously present fractures across the crown. Such fractures often yielded enamel-plus-dentine fragments, rather than enamel fragments separated from dentine. Some of the findings based on these 'thermal shock experiments' are:

a) Samples need to be air dried prior to treatment. Water-saturated samples will fracture upon transfer to liquid nitrogen. Although that was the objective of the treatment, fracturing tended to occur in places that were not desirable, such as across the crown itself, generating multiple enamel/dentine fragments. Alternatively -

b) The first step should be the hot plate to remove any remaining water.

c) Problems with non-molar teeth included burning of the root (because the root was in contact with the hot plate). The root then became very friable and made the whole sample difficult to handle.

Preparation of thick sections

An alternative method, suggested by Dr. J. Osborn (Dept. of Dentistry, Division of Oral Biology, Univ. of Alberta), is to prepare relatively thick sections from a tooth, and to use these sections for analysis with different techniques. Separating the enamel and the dentine is possible for such sections by using a dental drill.

Initial experiments on thick sections of teeth using tungsten-carbide burs did not yield satisfactory results. The burs created much vibration, which increased the risk of losing chips of enamel. In addition, the tungsten-carbide might leave a residue on the sample. A diamond-tipped dental drill fitted to a 430-K™ High Speed Handpiece, proved very suitable for the task of removing dentine. In addition, diamond (*i.e.*, carbon) cannot contaminate the sample with elements that interfere with the measurements. As illustrated in Figure 4.7, the roots were first removed using a separating disk ('improved red flash #200' Keystone – National Keystone Products Co.) in order to facilitate access to the dentine with the drill. The samples were checked regularly with a stereomicroscope in order to determine if all dentine had been removed. A Ziploc bag was used to prevent any chips of enamel from getting lost. The left hand holding the sample can be put in the bag which is then zipped close. The handpiece can be inserted into the bag through a hole made in one of the sides.

This method of sample preparation, *i.e.*, the preparation of thick sections, has the added advantage that it allows for analysis of one sample with both bulk and microanalytical techniques.

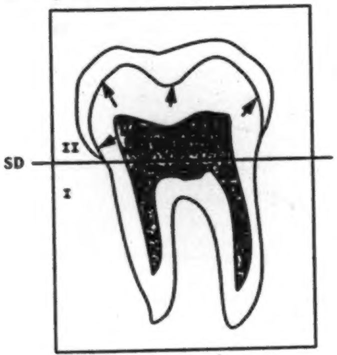


Fig. 4.7: Sample preparation method for NAA using a separating disk and bur. The separating disk is used to cut the tooth just below the CEJ (at level 'SD'). The roots (part I) are not further used in this study. The crown (part II) is further prepared by using the bur to remove the dentine in the direction of the arrows, until the enamel is reached.

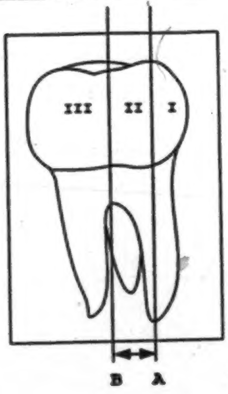


Fig. 4.8: Preparation of the permanent teeth: The samples are embedded in epoxy and sectioned in BL direction using a diamond blade saw. The first section (line A) goes through the highest point of the cusp. Part I is polished in preparation for microprobe analysis. Another section (line B) is made, so that a thick section of ca. 1.5-2 mm is obtained (part II). This section is further prepared for NAA (see Fig. 4.7). The remaining part of the tooth (part III; sectioned, unpolished) is used in LA-ICP-MS.

Preparation of permanent teeth

For the final analysis of the permanent teeth the following method was used (Figure 4.8): the samples were embedded in epoxy and sectioned in BL direction using a diamond blade saw. One thick section was prepared such that, for molars, the section would go through the highest points of the cusps (*i.e.*, including the maximum amount of increments). Adjacent to this cut, another thick section of *ca.* 1.5-2 mm was made. The first section was polished using Beuhler Gamma Micropolish Alumina 3B 0.05 µm on a polishing cloth (Tech Met Canada, Ltd) for microprobe analysis. The latter section was prepared for neutron activation analysis (NAA) with the dental drill as described above. The remaining part of the tooth (sectioned, unpolished) was later used in laser ablation ICP-MS. Seven of the maxillary teeth were selected for analysis with LA-ICP-MS: All the left teeth (P-LC, P-LP3, P-LP4, P-LM1, P-LM2, P-LM3) and one right molar (P-RM1). For P-LM2, the unpolished section was not considered suitable for analysis and the polished section was selected instead.

Preparation of deciduous teeth

The selection of samples from among the available deciduous teeth was partly determined by the fact that several of the teeth had broken in 2, 3 or even 4 parts due to desiccation and, probably, handling over time. In all, 17 samples were selected for embedding and sectioning (Table 4.8). Because these samples were to be analyzed with laser ablation ICP-MS only, they required minimal preparation.

The deciduous teeth were prepared in the Department of Archaeological Sciences at the University of Bradford (U.K.). The selected specimens were placed in reusable rubber mounting cups (Buehler - single specimen per cup) and two moulds with four different compartments, labelled with dots 1-4 to aid in identification of the samples during and after embedding. Prior to embedding, the moulds were sprayed with a releasing agent to facilitate release of the sample from the mould.

Table 4.8: Deciduous teeth selected for analysis. R= right, L= left; i1= central incisor, i2=lateral incisor, c=canine, m1=first molar, m2=second molar.

IND.	*Tooth*	*Label*	*Remarks*
A	Upper Li1	A-Li1	(NB: no canines available)
	Upper Ri2	A-Ri2	
	Upper Rm1	A-Rm1	Broken in 2 parts in BL direction
	Upper Rm2	A-Rm2	Broken in 2 parts in MD direction
B	Upper Ri1	B-Ri1	
	Upper Ri2	B-Ri2	2 parts
	Upper Rc	B-Rc	2 parts
	Upper Rm1	B-Rm1	
	Lower Rm2	B-Rm2	
C	Upper Ri1	C-Ri1	(NB: other canines and lateral incisors are too worn)
	Lower Lc	C-Lc	
	Upper Lm1	C-Lm1	
	Upper Lm2	C-Lm2	
D	Lower L?c	D-L?c	
E	Upper Li1	E-Li1	
F	Lower Li2	F-Li2	
	Lower Lm2	F-Lm2	

The samples were embedded in Buehler Epo-thin low viscosity epoxy resin and left to cure overnight at room temperature, taking approximately 18 hours to set. Subsequently, the specimens were cut on an ISOMET 11-1180 Low speed saw (Buehler Ltd) fitted with a diamond wafering blade running under IMS (Industrial Methylated Spirit) at speeds 4-5. After sectioning, the specimens were ultrasonically cleaned in deionized water and acetone, respectively, for ca. 1 minute. Samples which were embedded and did not need sectioning to acquire the appropriate plane for analysis were ground down with Buehler-met™ metallographic grinding paper (p240 and p600) to remove the resin and expose the dental tissues. Whereas careful polishing of the sample is essential in the case of electron microprobe analysis, it is not necessary in the case of laser ablation analysis[1]. Thus, for the deciduous specimens, final polishing was done only briefly to remove the cutting traces from the saw, using abrasive strips (Aluminium oxide) 30 µm and 3 µm. Following the polishing procedures all samples were cleaned ultrasonically in deionized water for ca. 5 minutes.

Analytical techniques

For a detailed study of the variation within and between teeth of the same individual, as well as between teeth of different individuals, both a bulk technique and a micro-analytical technique were selected.

Bulk analysis

Various techniques are available for this purpose, such as AAS, ICP-AES, and NAA (for a list of abbreviations see p. *iv*). The former two require the sample to be dissolved and thus involve elaborate sample preparation procedures during which contamination could be introduced. Additionally, relatively large sample sizes are required for AAS because various aliquots need to be prepared, one for each element of interest. In contrast, NAA is a multi-elemental technique that can be applied to liquids, solids and powdered samples. Neutron activation analysis was considered appropriate for our purpose. It is an affordable technique that requires relatively little sample preparation, and is available on-campus (SLOWPOKE Reactor Facility, Univ. of Alberta).

NAA

NAA requires a neutron source, generally a nuclear reactor, to irradiate the sample. When an atom in the sample captures a neutron, a process referred to as *neutron activation*, an isotope of that atom with a higher atomic weight is formed. Although some isotopes are stable, the majority are unstable and these undergo radioactive decay. During decay, different types of radiation (e.g., α, β, γ) with specific energies characterize each isotope. Analysis of the decay process can yield information about the type and amounts of the elements present in the sample.

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[1] In fact, it was found that the use of polished specimens for laser ablation reduced the number of counts significantly (permanent tooth sample LM2), because the polished, shiny surface works like a mirror and partly reflects the beam off the sample.

The technique has been successfully applied to both modern and archaeological bone and tooth samples, using a neutron flux varying from 1.10^{11} $n.cm^{-2}.s^{-1}$ to 1.10^{14} $n.cm^{-2}.s^{-1}$ (e.g., Brätter *et al.*, 1977; Wessen *et al.*, 1977; Badone & Farquhar, 1982; Geidel, 1982; Edward *et al.*, 1984; Hancock *et al.*, 1987, 1989; Vernois *et al.*, 1988a, b; Ung Bao *et al.*, 1990; Harritt & Radosevich, 1992). Table 4.9 shows the elements that could be detected in these studies (see also Appendix A for an explanation of the chemical symbols). In addition, some rare earth elements (e.g., europium (Eu) and lanthanum (La)), and uranium (U) have been detected in these materials.

Table 4.9: Periodic table of the elements. Elements detected in bone and teeth with NAA are indicated by shading (for references see text).

Group	1A	2A	3B	4B	5B	6B	7B	8X	8Y	8Z	1B	2B	3A	4A	5A	6A	7A	8A
Period																		
1	1 H																	2 He
2	3 Li	4 Be											5 B	6 C	7 N	8 O	▨ 9 F	10 Ne
3	▨ 11 Na	▨ 12 Mg											▨ 13 Al	14 Si	▨ 15 P	16 S	▨ 17 Cl	18 Ar
4	19 K	▨ 20 Ca	▨ 21 Sc	▨ 22 Ti	▨ 23 V	▨ 24 Cr	▨ 25 Mn	▨ 26 Fe	▨ 27 Co	28 Ni	▨ 29 Cu	▨ 30 Zn	31 Ga	32 Ge	▨ 33 As	34 Se	▨ 35 Br	36 Kr
5	37 Rb	▨ 38 Sr	39 Y	40 Zr	41 Nb	▨ 42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	▨ 51 Sb	52 Te	53 I	54 Xe
6	55 Cs	▨ 56 Ba	LA	72 Hf	73 Ta	▨ 74 W	75 Re	76 Os	77 Ir	78 Pt	▨ 79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
7	87 Fr	88 Ra	AC															

In the first pilot study using INAA (Instrumental Neutron Activation Analysis[2], hereafter simply referred to as NAA), one test sample of enamel, weighing 37.4 mg, was irradiated for 4 minutes (short irradiation; for short-lived radioisotopes), and for 2 hours (long irradiation; for long-lived radioisotopes) at a neutron flux of 1.10^{12} $n.cm^{-2}.s^{-1}$. From the short irradiation, information on levels of Na, Cl, Ca, Sr, Mn, and Mg could be obtained.

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[2] INAA refers to neutron activation without chemical preparation of the sample (Potts, 1987).

The long irradiation provided information on Na, Ca, bromine (Br), Zn, Sr, gold (Au) and cobalt (Co) levels, and indicated the possible presence of Cd. The radioisotope ^{32}P of phosphorus, one of the major constituents of enamel, decays by emitting pure, high-energy β-radiation. This produces intense low energy background radiation, which hinders the detection of a number of elements.

The neutrons that are produced in the reactor can be divided into three types:

a) *thermal neutrons*, with a relatively low energy (up to 0.5 eV);

b) *epithermal neutrons*, with an intermediate energy (0.5 eV-1 MeV). This can be used to selectively activate certain isotopes;

c) *fast neutrons*, with a high energy (> 1 MeV), which tend to produce interfering reactions (Parry, 1991).

Epithermal Neutron Activation Analysis (ENAA), uses a boron carbide shield to absorb the thermal neutrons, while letting the epithermal neutrons reach the sample. This reduces the activation of Na, Cl, and Mg, thereby facilitating the detection of Sr, Ba, U and the rare earth elements (REEs). The advantages of ENAA were tested on a series of samples that were analyzed to study intra- and inter-tooth variability (see below).

The SLOWPOKE Reactor uses a relatively low neutron flux (up to a maximum of 1.10^{12} n.cm^{-2}.s^{-1}) compared to some of the studies mentioned above. Therefore, some of the elements of interest were not detected using this technique. Sample size is one of the factors determining the detection limits. Because deciduous teeth are smaller in size and have thinner enamel than permanent teeth, it was decided that NAA not be applied to the deciduous tooth samples since amounts of enamel yielded would most likely be insufficient.

Sampling strategy and analytical procedures

Bulk analysis was to be applied to sections of enamel, not whole crowns. Therefore, some test samples were prepared to determine the level of intra- and inter-tooth variation in trace element composition. Modern samples (a lower canine and an upper molar) were selected from the specimens obtained from the Oral Surgery Clinic (Univ. of Alberta).

The molar was sectioned longitudinally in a buccolingual (BL) direction (samples T-M3-1, T-M3-2, T-M3-3, T-M3-4; Fig 4.9a) and the canine was sectioned in the horizontal (occlusal) plane (samples T-C_1 and T-C_2; Fig 4.9b). The 3rd portion, near the root, was found to contain insufficient amounts of enamel for NAA and was not included in the analysis. The obtained sections for the test samples, as well as the permanent maxillary teeth, were further prepared with a diamond-tipped dental drill as described above (*Preparation of thick sections*, Fig. 4.7). Sample weights for the test samples and permanent tooth samples are given in Table 4.10.

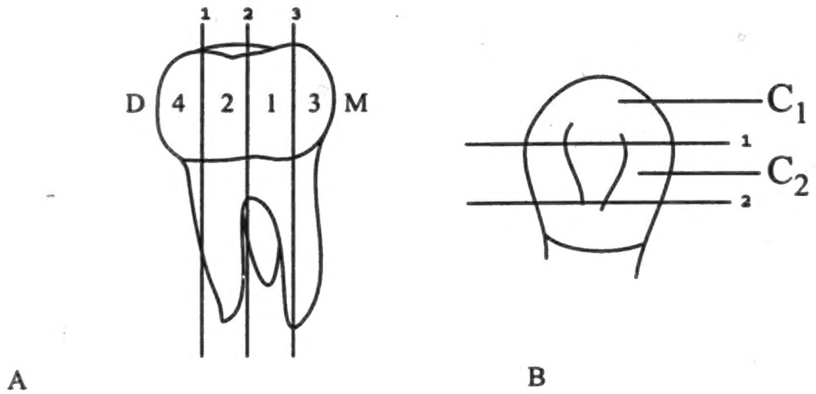

Fig. 4.9. A: The molar was sectioned longitudinally in a buccolingual (BL) direction to yield four samples (T-M3-1, T-M3-2, T-M3-3, T-M3-4). M=mesial, D=distal. B: The canine was sectioned twice in the horizontal (occlusal) plane, resulting in samples T-C_1 and T-C_2. The third portion, near the root, was not included in the analysis.

Additional samples included an enamel fragment of an archaeological specimen from the Netherlands (sample: 'T-Dist Orig'), and from a ca. 8000 year old tooth from Algeria (sample T-3A-3). Results of the pilot studies are included in Chapter 5, where the results of NAA and LA-ICP-MS of the final samples are given.

All samples were rinsed twice with distilled, deionized H_2O and once with ethanol (95%), air-dried and weighed into nitric acid washed 0.75 ml (cut) polyethylene vials. For analysis of the test samples, two samples of different weights (0.0382 g and 0.0636 g) were prepared for the standard (NBS 1633a), because the sensitivity of the standard under the given analytical circumstances was unknown beforehand. The smaller sample turned out to be very active, and the 0.0636 g sample was therefore not included in the analysis. The NBS standard was counted twice to check for reproducibility. A europium (Eu) standard was included for energy calibration.

*Table 4.10: Left side of the table: Sample IDs and mass of the test samples analyzed to determine intra- and inter-tooth variability. Right side: Sample IDs and mass of enamel samples from the maxillary permanent teeth prepared for NAA by removal of dentine from the section using a diamond-tipped bur. See Table 4.1 for description of samples. * NBS = National Bureau of Standards. 1633a consists of trace elements in local fly ash. **SO-2: a soil standard prepared by Canada Centre for Mineral and Energy Technology – CANMET.*

ID	Net Mass (mg)	ID	Net Mass (mg)
T-C_1	76.4	P-LC	47.8
T-C_2	72.3	P-LP3	47.9
T-3A-3	93.3	P-LP4	67.1
T-Dist. Orig.	84.8	P-LM1	60.5
T-M3-1	90.4	P-LM2	77.6
T-M3-2	94.5	P-LM3	54.8
T-M3-3	150.0	P-RP3	45.4
T-M3-4	141.0	P-RP4	64.0
NBS 1633a*	38.2	P-RM1	82.3
SO-2**	83.9	P-RM2	104.4
		P-RM3	54.0

- *Analysis of short-lived radionuclides*

Short-lived radionuclides were analyzed with NAA using short irradiation (240 seconds) at a flux of 1.10^{12} n.cm^{-2}.s^{-1}. Two counts were carried out: a first count immediately after irradiation with a sample-detector distance of 10 cm. The only exception was sample T-M3-3, which was counted after a decay time of 4 minutes due to its weight (150 mg). A second count was made after a decay period of *ca*. 45 minutes (when short-lived isotopes have decayed), using a sample-detector distance of 6 cm, a 19% hyperpure Ge detector, and a counting period of 600 seconds.

- *Analysis of long-lived radionuclides*

Long-lived radionuclides were determined with NAA using a 2 hour irradiation period at a flux of 1.10^{12} n.cm^{-2}.s^{-1}. Counting was undertaken for 50,000 seconds after decay periods of 6 days and 6-8 weeks, with a sample-detector distance of 1 cm. For the first count of the test-samples (after 6 days), a 0.5 mm Pb sheet was used to reduce the ^{32}P bremsstrahlung (background radiation). However, the Pb sheet did not appear to make a significant difference, and it was not used again for the second count after 6-8 weeks, or for the maxillary samples. During the first count another standard was used as well: SO-2 (a soil standard prepared by Canada Centre for Mineral and Energy Technology - CANMET).

Table 4.11 shows the short-lived and long-lived radionuclides that have been used for the determination of elemental concentrations in the samples.

Table 4.11: Radionuclides used for determination of the concentrations of elements, showing their specific gamma-ray energies and half-lives ($T_{½}$). m=minute, h=hour, d=day, y=year. Source: Browne & Firestone (1986).

Element	*Radionuclide*	*Gamma ray(s) (keV)*	$T_{½}$
Short-lived radionuclides			
Na	^{24}Na	1368.6	14.66 h
Cl	^{38}Cl	1642.7 2167.7	37.24 m
Mn	^{56}Mn	846.8	2.578 h
Sr	^{87m}Sr	388.4	2.795 h
Long-lived radionuclides			
Sc	^{46}Sc	889.2 1120.5	83.83 d
Fe	^{59}Fe	1099.2 1291.6	44.496 d
Co	^{60}Co	1173.2 1332.5	5.27 y
Zn	^{65}Zn	1115.5	244.1 d

Data analysis

The NAA data are based on counting statistics and are given as average concentrations ± one standard deviation. To test the significance of the difference between means the following formula was used:

$$(X_1 - X_2) / \sqrt{(\sigma_1^2 + \sigma_2^2)}$$

in which (X_1-X_2) is the difference between means, and $\sqrt{(\sigma_1^2+\sigma_2^2)}$ is the estimated s.d. of this difference. Accordingly, the difference is expressed in s.d. units, and the probability is derived from statistical tables for the standard normal distribution.

Microanalysis

One type of microanalytical technique is based on X-ray fluorescence spectroscopy (XRF). In general, these techniques use the following principle: A primary beam is used to excite the sample and thus induce the emission of secondary X-rays. Each element produces X-rays with one or more characteristic wavelengths. The concentration of an element in the sample can then be determined by measuring the X-ray intensity at the characteristic wavelength for that element with either an energy dispersive spectrometer (EDS), or a wavelength dispersive spectrometer (WDS).

SEM/EDS

Schneider (1984, 1986) applied SEM/EDS to enamel samples from six prehistoric populations of Amerindians from Ohio. In her study, she was able to detect Ca, P (major elements), Mg, Mo, Cu, Mn, Se, Zn, Fe, Ni (trace essential), Sr, Al (non-essential) and Pb (toxic). In an attempt to reproduce her findings, I carried out several pilot studies with modern and archaeological samples using an SEM/EDS system (SEM: Hitachi S-2700; EDS system: Link eXL with a Pentafet lithium drifted Si detector - Dept. of Chemical and Materials Engineering, Univ. of Alberta). The aim of these studies was to determine which elements could be detected, at what concentrations the selected elements occurred, and whether there was any particular distribution of elements within the enamel. Most of the elements used by Schneider were found to be below or around detection limits. The peaks that could most clearly be distinguished were for the elements: Ca, P, Na, Mg, K, Cl, Al, Si and S. Several of the elements of interest from a palaeodietary perspective (e.g., Sr, Ba, Cu) could not, or not reliably, be detected with this technique. Therefore, additional pilot studies were carried out using the more sensitive electron microprobe.

Electron probe microanalysis (EPMA)

The microprobe used in this study, a JEOL Superprobe JX-A-8900R WD/ED combined microanalyzer (Department of Earth and Atmospheric Sciences, Univ. of Alberta), has five different wavelength dispersive spectrometers in addition to an energy dispersive spectrometer. This makes it possible to measure up to five elements simultaneously. Prior to analysis, the elements of interest are selected and a calibration against suitable standards of known composition is performed (see also Appendix C).

With regard to sample preparation procedures, the only requirement is that the sample has a polished surface. Ideally, the surface should be flat on a microscale. Polishing irregularities, in the form of scratches or a slightly tilted surface, will affect the measurements.

In order to study the variation in trace element composition within tooth crowns, the sampling strategy consisted of linear transects across the tooth, both in a cross-sectional direction (dentine to outer enamel across the EDJ) and longitudinal direction (top of crown towards CEJ, following the developmental time axis of the tooth; Fig. 4.10). In addition to the elements that could be detected with SEM/EDS (Ca, P, Na, Mg, K, Cl, Al, Si and S), samples were analyzed for F, Ba, Sr, Zn, and Fe.

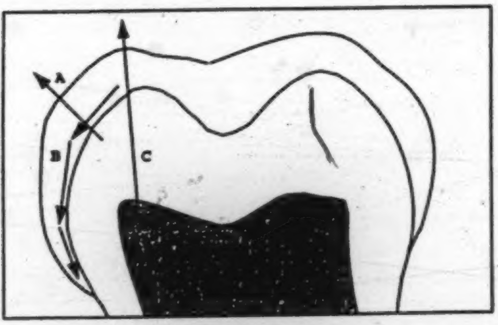

Fig. 4.10: The sampling strategy with the microanalytical techniques: linear trajectories across the tooth are analyzed, both in a cross-sectional direction (lines A and C) and longitudinal direction (line B). The longitudinal lines often consisted of two or three separate lines, following the curvature of the EDJ.

Some results from the microprobe analysis are included in Chapter 5. It was decided, however, that a more sensitive technique was needed for the microanalysis of the samples. Of the possible alternatives, laser ablation ICP-MS was considered the most promising option. It is one of the more recently developed techniques and offers rapid multi-elemental analysis of solid samples with high sensitivity and requires little or no sample preparation.

LA-ICP-MS

In laser ablation ICP-MS, the sensitivity of the ICP-MS instrumentation is combined with a laser system for sample introduction. One of the main advantages of this technique is that it can be directly applied to solid samples with little or no sample preparation (no extensive polishing necessary). The elaborate sample preparation processes - with concomitant risks of contamination - required for the more traditional fluid sample introduction methods, is thereby avoided. Additional advantages compared to fluid sample analysis include the possibility to study the spatial distribution of trace elements within the sample (using either points, line scans or areas), and the high speed with which the analyses are carried out.

The analysis takes place in three separate units:

1) Laser ablation takes place in the specimen chamber ('sample cell'). The energy of the laser beam reaching the sample surface is very rapidly converted into heat and the area targeted by the beam is vaporized. During this process atoms, ions, molecular species and particulate matter are generated. The ablated material is carried out of the sample cell by a carrier gas (usually argon) towards the ICP-unit;
2) In the ICP-unit the sample material is introduced into a hot argon plasma (~ 8000 K) and all the particles are atomized and ionized;
3) Finally, the ions are separated by atomic mass and charge in the mass spectrometer.

The nature of the interaction between laser and sample ('coupling of laser to sample') partly depends on the matrix characteristics. Therefore, for full quantification of data, sample and standard should be matrix-matched. Certain matrices, such as the apatite matrix of bones and teeth, are very difficult to match. In the absence of a suitable reference, glass standards (e.g., NIST SRM 610, 611, 612, 613) are often used for the calibration of such samples (Durrant, 1999). Several researchers have discussed the problems with full quantification of compositional data from bone and teeth (e.g., Cox *et al.*, 1996) and they recommend the use of a major element (Ca, P) for normalization or the use of inter-element ratios. For this study, comparisons of trace element levels between and within teeth are required (*i.e.*, relative differences), so that the restriction of semi-quantitative data does not represent a significant problem.

Examples of LA-ICP-MS studies of teeth include the monitoring of heavy metal exposure by sampling cementum growth layers in walrus teeth (Evans *et al.*, 1995) and by comparing pre- and post-natal enamel of human deciduous teeth (Lee *et al.*, 1999), the migration of dental amalgam components in human teeth (Hoffmann *et al.*, 2000), and the fingerprinting of individual teeth (Cox *et al.*, 1996). As is demonstrated by these and other studies, this technique is highly suitable for the purpose of determining intra-tooth variation in trace element composition. Line scans as well as two-dimensional elemental distribution maps can be performed relatively quickly, which is an important advantage over electron microprobe analysis.

Sampling strategy and analytical procedures

- *Selection of elements*

Eleven elements were selected for analysis: the major elements Ca and P, the essential elements Cu, Fe, Mn, Mo, V, and Zn, and the non-essential/toxic elements Ba, Sr, and Pb. Most elements have multiple isotopes, and the selected isotope for each element, and its relative abundance in nature, are listed in Table 4.12.

Initially, carbon (as ^{13}C) was included instead of P, to gain insight into the distribution of organic material, and organically associated trace elements, within enamel. However, the results of the first test-runs using three of the permanent teeth (P-LC, P-LP3, P-LM3) indicated that background counts for carbon were so high that this element provided virtually no information. Likewise, ^{56}Fe was dropped in subsequent analyses as its atomic mass (amu) is similar to that of argon oxide (ArO - which is abundant because argon is used as the carrier gas), causing isobaric interference.

Initially NIST SRM 612 was used as an external (glass) standard. However, as was mentioned above, apatite and glass have a very different matrix. For this reason several studies recommend the use of major elements as internal standards. Calcium and phosphorus were included for this purpose and data were analyzed in a semi-quantitative fashion only, with results for each isotope expressed as a Ca-ratio.

Table 4.12: A list of elements selected for LA-ICP-MS, showing the abundance of each isotope and its atomic mass (amu).

[illegible]	[illegible]	[illegible]
^{13}C	1.10	13.00
^{31}P	100.00	30.97
^{46}Ca	0.004	45.95
^{51}V	99.75	50.94
^{55}Mn	100.00	54.94
^{57}Fe	2.2	56.94
^{65}Cu	30.83	64.93
^{66}Zn	27.9	65.93
^{88}Sr	82.58	87.91
^{95}Mo	24.13	97.91
^{138}Ba	71.70	137.91
^{208}Pb	52.4	207.98

- *Analytical procedure*

The analyses were performed at the Centre for Analytical Sciences (Univ. of Sheffield, U.K.). The equipment consisted of a CETAC LSX-200 Laser Ablation System coupled to a Hewlett Packard HP4500 ICP-MS system. The system uses a Nd:YAG frequency quadrupled laser operating in the UV range at 266 nm. The system was tuned with a fluid sample using mass to charge ratios (m/z) 59 (Co), 139 (La), and 232 (Th).

The sample is placed in the specimen chamber. Although it need not be polished flat, the sample surface should be even and oriented perpendicular to the beam. Once the sample is mounted, it can be visualized on a monitor by adjusting the XYZ stage to bring the sample into focus. In order to define line scans, the starting point and end point of such lines were programmed using the control software. Parameters that can be controlled via the software are the laser pulse repetition rate, the energy and spot size of the beam, and the scan speed. Figure 4.11 is a schematic representation of the set-up of the laser ablation ICP-MS system.

Continuous line scans were performed across the teeth in various directions covering both enamel and dentine. Although enamel is more resistant to diagenesis, and therefore of more interest in applications of this technique to archaeological samples, this study was exploratory and dentine was also included in the analysis.

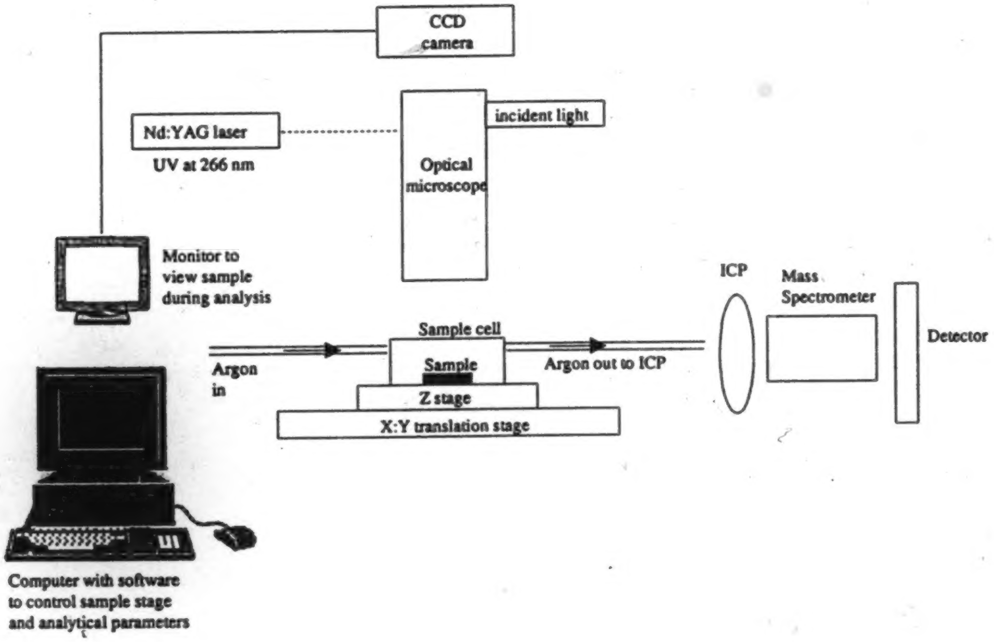
CCD camera
incident light
Nd:YAG laser
UV at 266 nm
Optical microscope
Monitor to view sample during analysis
ICP
Mass Spectrometer
Detector
Sample cell
Sample
Argon in
Argon out to ICP
Z stage
X:Y translation stage
Computer with software to control sample stage and analytical parameters

Fig. 4.11: Schematic representation of the set-up of the LA-ICP-MS system. The sample is placed in the sample cell, which is continuously flushed with an argon carrier gas. Through the optical microscope and CCD camera the sample surface is displayed on a monitor. Adjustments to sample position and focussing can be done by moving the XYZ stage. Sample material, which is removed by pointing the laser at the surface, is carried to the ICP-unit through a tube system, and subsequently analyzed in the mass spectrometer.

The system was operating in "Q-Switched", time resolved mode (TRA)[3]. A laser pulse repetition rate of 10 Hz with an energy level of 4.0 mJ/pulse was used, with a spot size of 150-200 μm. The scan speed was set to 40 μm/s, except for the mapping of one deciduous molar from individual C (sample C-Lm2; scan speed 50 μm/s) and line 5 on sample F-Lm2 (scan speed 10 μm/s). Integration time for the highly abundant P (relative abundance 100%) was adjusted to 0.01 s to prevent detector overflow (set to 0.1 s for the other elements). Typically, relative standard deviations (RSDs) are in the order of 8-12% (A. Cox, *pers. comm.*).

Prior to collection of data from the sample, a gasblank was counted for ca. 20 seconds. Once the laser is switched on, the beam starts to progress across the sample according to the predefined path. The signal takes a few seconds to stabilize, while the counts for various elements continue to increase. The counts for each element are presented on the screen in real time, so that the behavior of elements can be monitored during the analysis.

There is a delay of ca. 5 seconds in the signal because the ablated material must travel through the tube system from the sample chamber to the ICP and then on to the detector. A similar delay was also observed when the beam was crossing a boundary region, such as from epoxy into enamel or *vice versa*, or across a crack in the tooth. When the laser is turned off, it takes some time before the counts are back to their gasblank levels. Because the average number of counts for the different isotopes differs considerably, this decay (or 'memory effect') is of variable duration depending on the isotope.

In general, the behaviour of the elements across boundary regions is not easy to understand because several things happen at the same time: for example, when the laser moves from the epoxy into the enamel, the high calcium ratios of some elements in the outer enamel region are partly real (as can be seen from the actual cts/sec for the isotope), and partly due to the fact that counts for Ca take longer to reach their high levels.

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[3] The system can operate in two modes: 'free-running' or 'fixed-Q' mode, in which a single laser pulse is produced, and 'Q-Switched' mode, whereby the oscillation of the laser between two mirrors is blocked. The stored energy is then released in a short pulse of much higher power than can normally be generated. In this mode, a large and more shallow crater is produced. In addition, because of improved ablation efficiency, the sample tends to be more representative of the sample composition (Durrant, 1999). Multiple pulses can be generated in order to obtain a relatively constant signal. In this way, by moving the laser beam across the sample, line scans can be performed. In this case individual craters overlap to create a linear, straight-walled channel.

In 'Time Resolved Analysis' (TRA), the sample is continuously ablated while the beam is progressing across the sample at a certain speed. The data is collected in particular time units (counts/second) (Lee *et al.*, 1999).

The raw data files are pre-processed by the software included with the laser ablation system. The original signal is transformed so that the continuous data eventually are presented in a spreadsheet file as data 'points' (counts/second for each element) along the length of the trajectory. These spreadsheet files, in which columns correspond to the different isotopes and rows are counts/second for each isotope along the laser trajectory, are then available for further analysis.

- *Visual inspection of laser trajectories*

After analysis, the seven samples from the maxillary permanent dentition (P-LC, P-LP3, P-LP4, P-LM1, P-LM2, P-LM3, P-RM1), and the 18 deciduous samples (17 samples including the specimen from which the 2D-distribution maps were prepared) were taken to the SEM-lab in the Dept. of Chemical and Materials Engineering (Univ. of Alberta). All laser trajectories were visualized using a Hitachi S-2700 SEM operated at 10kV. Digitized images (.BMP; 8 bit, 1024 x 816) were collected with a PGT IMIX system. From all the separate images of one tooth, a composite image was created (using Star Office Drawing on Linux) to obtain an overview of the specimen and the location of the laser trajectories (see Appendices D and E). These were used to aid in interpretation of the laser ablation data sets.

- *Data processing and analysis*

For all data files, the gas blank and measurement areas were first determined, using scatter plots based on the original data set, as well as notes made during the analysis (the countdown on the computer screen was used to mark certain events). The original data files were processed using a Fortran program (*LADA;* Laser Ablation Data Analysis) specifically written for this purpose by B. Hazes. Among the options offered by *LADA* are:

- Subtracting the gas blank value from all measurement values
- Analysis of user-specified ranges

- Calculation of Ca-ratios (with Ca set to 100,000) and ratios based on user-specified elements
- Calculation of correlations between different elements
- Histograms based on normal probability, displaying the data points within –3.5 to +4.0 s.d. units
- Removal of outliers
- Calculation of basic statistics (average, s.d., s.e., min., max.)
- Calculation of logarithms
- Writing data to a new output file

All data were transformed into Ca-ratios prior to further analysis. *LADA* was used to explore the data sets, and to create restricted data files based on particular areas of the teeth (for example, upper crown region, or lower crown region). From the resulting data sets, plots were created for use in the initial assessment of variability and behaviour of each element and of the element/Ca ratios across the transects (for example Figures 5.7-5.13). These plots were created in the following way:

1) Select the appropriate data range;
2) Calculate average gasblank element counts and subtract them from the data;
3) Calculate Ca-ratios;
4) Detect and flag outliers (> 3 s.d.);
5) Replace flagged outliers with estimates based on linear interpolation between valid nearest neighbours;
6) Calculate moving averages using a window of 5 to smooth the signal;
7) Create plot files for the graphs.

The plot files were processed into graphs by the program RESPLT (H. Schreuder, unpublished results). For further analysis of the data files, LADA, a spreadsheet program and the *SPSS* statistical package (version 10.0) were used.

CHAPTER 5

RESULTS & DISCUSSION

Stable isotope analysis of dental tissues has been applied to the study of weaning age in prehistoric populations. For such studies, bulk analysis of different teeth from the same individual, formed at different periods of development, are used to determine at what approximate age the change in diet occurred.

Studies of intra-tooth variation in chemical composition can potentially provide more precise information than inter-tooth bulk analysis about when dietary changes occurred in the life of an individual. Theoretically, the fact that enamel in different parts of a tooth is deposited at different points in time suggests that changes in diet may also be recorded within a single tooth. Accordingly, using a microanalytical technique, it is possible to study changes in chemical composition along lines following the developmental axis of a tooth. Knowledge of variation in trace element concentrations is also important to judge to what extent a small sample (for bulk analysis) is representative for the chemical composition of a tooth.

In this study, the bulk and microanalysis of multiple teeth from several dentitions allowed us to determine the extent of variation in trace element composition at various levels:

1. within a single tooth;
2. between teeth of the same type of a single individual;
3. between different tooth types of a single individual, and
4. between teeth of different individuals.

In addition, the trace element composition of the deciduous teeth could be compared with the available dietary records.

When the present study was initiated, the use of a microanalytical technique to study patterns in trace element concentrations in enamel was a novel approach, although some earlier studies had been carried out based on the idea that different areas within enamel reflect different stages of enamel deposition (e.g., using sequential etching of surface layers of enamel). Therefore, some pilot studies were carried out to determine which technique was the most suitable, which trace elements could be included in the analyses (this would depend on the selected technique), and which trace elements showed variation along a trajectory in enamel from the cuspal/incisal area to the CEJ.

Bulk analysis

Neutron Activation Analysis

Pilot studies (inter- and intra-tooth variation)

Eight samples were analyzed with instrumental NAA to test for intra- and inter-individual differences, using both short-lived and long-lived radionuclides (see Chapter 4, p. 104: Sampling strategy and analytical procedures). The samples included an archaeological specimen (T-3A-3; Algeria), a modern sample ('T-Dist.Orig.'), an upper and middle section of a modern canine (T-C$_1$ and T-C$_2$, respectively) and four longitudinal slices of a modern third molar (T-M3-1 to T-M3-4; see Fig. 5.1). The sections of the modern canine and third molar were included to determine if and how the results of a bulk analysis depend on the area of the tooth that is sampled. The results are given in Tables 5.1 and 5.2.

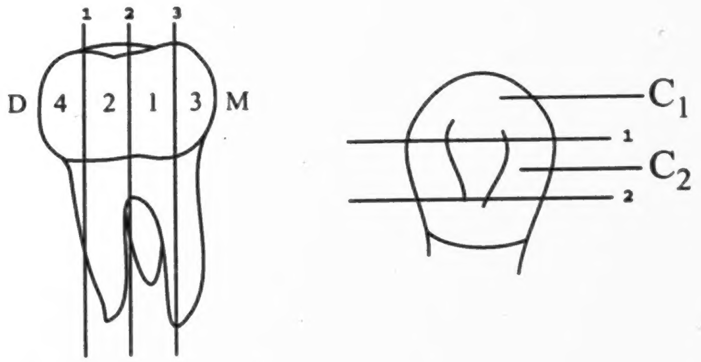

Fig. 5.1: Schematic diagram showing the preparation of the molar and canine for NAA. The molar was cut three times to yield four sections of similar thickness (sample 1-4). The canine was cut twice to yield three horizontal sections. Samples T-C$_1$ and T-C$_2$ were analyzed, but the section closest to the root was not included.

Table 5.1: Results from the analysis of the test samples (short-lived radionuclides). Concentrations are given in ppm (= μg/g) ± 1σ. Blk= blank; 1633a is the standard, consisting of trace elements in local fly ash.

ID	wtd mean % Na*	wtd mean Cl *	Mn	Sr
T-M3-1	7410 ± 30	2720 ± 30	0.78 ± 0.12	186 ± 18
T-M3-2	7540 ± 30	2650 ± 30	0.69 ± 0.09	188 ± 18
T-M3-3	6930 ± 30	2960 ± 30	0.82 ± 0.08	190 ± 14
T-M3-4	6880 ± 30	2970 ± 30	0.83 ± 0.10	165 ± 17
T-C_1	7150 ± 40	2960 ± 40	1.03 ± 0.12	113 ± 19
T-C_2	7190 ± 40	2830 ± 40	1.03 ± 0.13	166 ± 20
T-3A-3	6420 ± 30	3040 ± 40	6.20 ± 0.17	239 ± 18
T-Dist Orig	6590 ± 30	3070 ± 40	3.24 ± 0.16	81 ± 17
1633a	1700 ± 30	50 ± 20	171 ± 1	721 ± 46
1633a	1690 ± 30	40 ± 20	171 ± 1	811 ± 49
Blk	0.0 ± 0.0	0.0 ± 0.0	0.008 ± 0.0015	-0.036 ± 0.23

** For Na and Cl weighted means are based on concentrations as measured from ^{24}Na, keV-lines 1368.6 and 2754.0, and ^{38}Cl as measured from keV-lines 2167.5 and 1642.4.*

Table 5.2: Results from the analysis of the test samples (long-lived radionuclides). Concentrations are shown ± 1 σ: scandium and cobalt in ppb (= μg/kg) and zinc and iron in ppm (= μg/g). Values below detection limit are indicated by ' ≤ '.

ID	Sc	Co	Zn	Fe
T-M3-1	7.4 ± 1.3	60 ± 13	143 ± 14	≤ 43
T-M3-2	3.6 ± 1.1	≤ 43	121 ± 12	≤ 26
T-M3-3	5.1 ± 1.6	≤ 33	152 ± 15	≤ 22
T-M3-4	≤ 4.3	43 ± 11	132 ± 13	≤ 21
T-C_1	≤ 9.7	≤ 86	156 ± 15	≤ 38
T-C_2	9.6 ± 1.5	127 ± 18	140 ± 14	≤ 50
T-3A-3*	18.8 ± 1.5	102 ± 15	76 ± 8	≤ 30
T-3A-3	16.2 ± 1.5	100 ± 15	79 ± 8	≤ 42
T-Dist Orig	≤ 4.9	116 ± 14	190 ± 19	≤ 57

**Sample T-3A-3 was counted twice on consecutive days for check for reproducibility in the counting step.*
Sample T-3A-3 has 2.5 ± 0.1 μg/g Sb.
In sample T-C_2 Ba was detected at ≤ 336 ppm.

In general, the concentrations of trace elements in samples from the same tooth are more similar than the elemental concentrations in samples of different teeth. For the short-lived radionuclides measured in T-M3, the sections 1 and 2 (the two middle sections – see Fig. 5.1) are very similar in their composition, and the same is true for sections 3 and 4 (the two outer sections). However, a comparison of sections 1 and 2 with 3 and 4 shows statistically significant differences in the concentrations of Na and Cl. Although less extreme, the Sr concentrations for T-C_1 (113 ± 19 ppm) and T-C_2 (166 ± 20 ppm) differ significantly ($p < 0.05$). In this case the difference may reflect a biogenic signal since T-C_1 (the top section of the canine) is formed before T-C_2 (the middle section). Generally, Sr intake increases with the introduction of solid foods, which could have contributed to the higher levels in T-C_2. Similarly, Ba could be detected in sample T-C_2 (≤ 336 ppm) but not in T-C_1.

Sodium and Cl levels are relatively constant for all samples with the highest and lowest values differing by less than 20%. Based on their literature review, Curzon & Cutress (1983) report average values of 7100 and 3200 ppm for Na and Cl, respectively. The values for Cl found in this study are somewhat lower, but values as low as 720 ppm have been reported (Curzon & Cutress, 1983). Inter-tooth variation is considerably higher for the other elements. The Mn concentrations are comparable for the subsamples from the molar (T-M3) and canine (T-C), but variable among the different samples. Concentrations of this element vary nearly nine fold with the highest levels found in the prehistoric Algerian specimen T-3A-3 (6.20 ± 0.17 ppm). Diagenesis may be a factor but the observation of elevated Mn levels in the modern sample 'T-Dist. Orig.' (3.24 ± 0.16 ppm), and samples to be discussed later (see Table 5.3), indicates that high Mn concentrations can also be found in contemporary samples.

The variability in Mn concentrations found in this study agrees with literature reports (Losee *et al.*, 1974a; Curzon & Cutress, 1983). The prehistoric Algerian sample also contains the highest levels of Sr (239 ± 18 ppm) and Sc (16.2 –18.8 ppm). In contrast, it has the lowest concentrations of Na (6420 ± 30 ppm) and Zn (76-79 ppm). Finally, this was the only sample in which antimony (Sb) could be measured (2.5 ± 0.1 ppm). Antimony is normally found at very low levels and, as with our other samples, can often not be detected. In six studies, reviewed by Curzon & Cutress (1983), Sb was reported as ranging between 0 and 3 ppm (median 0.1 ppm). Rasmussen (1974) determined the concentrations of Sb, As, Br and Hg in samples of both ancient and contemporary teeth using neutron activation. For Sb, the levels ranged from < 0.001 - 1.59 ppm. The highest value (1.59 ppm) was found in samples dating to the Middle Ages.

In all other samples, concentrations ranged between <0.001 - 0.091 ppm, which suggests that 2.5 ppm for the Algerian sample is noteworthy.

Not much is known about Sb levels in individual foods (Nielsen, 1986). Furr *et al.* (1979) reported relatively high levels of Sb in various nuts (50-300 ng/g dry weight *i.e.*, 0.05 - 0.3 ppm). The highest concentrations were found in cashew, pecan and acorns. However, it is extremely difficult, if not impossible, to draw conclusions about diet without any contextual information for the individuals from which the samples were derived. In many palaeodietary studies, nuts have provided a "convenient caveat for seemingly inexplicable (*i.e.* high) element levels in bone" (Buikstra *et al.*, 1989: 183).

These initial NAA studies show that there is considerable inter-tooth variation in at least some elements and that, in general, intra-tooth variation is less pronounced. Data quality is excellent for Na and Cl, and good for Sr, Mn and Zn. The results for Sc, Co and Fe are less accurate. The canine sample provided a first indication that compositional variation for enamel deposited at different stages of development may occur. Changes in trace element composition during development can also be studied by looking at teeth from a single individual that are formed during different time periods. Such data will also reveal if the inter-tooth variation shown above for teeth of different individuals is also observed within a single individual.

Inter-tooth variation within a single dentition

The permanent teeth from a maxilla of a ca. 15-year-old individual (archaeological specimen) were analyzed with NAA (see Chapter 4, p. 84: Description of samples – permanent teeth). Eleven teeth were available for analysis; the left canine, premolars and molars, and the right premolars and molars. The sets of left and right teeth form a developmental time series, whereas a comparison between antimeres (e.g., P-LP3 and P-RP3) provides information about compositional variability of teeth formed during approximately the same time period. The results of the measurements are given in Tables 5.3 and 5.4.

Table 5.3: Results from the analysis of the maxillary teeth from the archaeological specimen from the Netherlands for short-lived radionuclides. All concentrations are given in µg/g (ppm) ± 1σ.

Sample ID	Na	Cl	Mn	Sr
P-LC	6830 ± 70	2620 ± 60	1.62 ± 0.16	76 ± 23
P-LP3	6030 ± 60	2670 ± 60	4.63 ± 0.21	71 ± 22
P-LP4	6840 ± 60	2600 ± 50	2.14 ± 0.16	110 ± 20
P-LM1	6830 ± 60	1920 ± 50	6.00 ± 0.23	57 ± 20
P-LM2	6780 ± 50	2300 ± 40	3.00 ± 0.16	74 ± 18
P-LM3	6790 ± 60	2690 ± 50	1.65 ± 0.17	58 ± 21
P-RP3	6710 ± 70	2880 ± 60	1.45 ± 0.17	90 ± 24
P-RP4	7010 ± 60	2680 ± 50	2.33 ± 0.16	96 ± 20
P-RM1	6730 ± 50	2140 ± 40	1.69 ± 0.12	97 ± 17
P-RM2	6710 ± 50	2410 ± 40	1.27 ± 0.12	136 ± 17
P-RM3	6910 ± 60	2790 ± 60	1.54 ± 0.16	74 ± 18

Table 5.4: Results from the analysis of the maxillary teeth from the archaeological specimen from the Netherlands for long-lived radionuclides. Concentrations for scandium and cobalt are given in ppb (= µg/kg) and zinc and iron in ppm (= µg/g). Values below detection limit are indicated by ' ≤ '.

Sample ID	Sc	Co	Zn (± 1σ)	Fe
P-LC	≤ 8.0	≤ 130	128 ± 3	≤ 79
P-LP3	≤ 9.0	≤ 160	114 ± 3	≤ 87
P-LP4	≤ 6.0	≤ 120	91 ± 3	≤ 66
P-LM1	≤ 9.0	≤ 140	65 ± 2	≤ 51
P-LM2	≤ 5.0	≤ 80	85 ± 2	≤ 57
P-LM3	≤ 7.0	≤ 150	92 ± 3	≤ 87
P-RP3	≤ 9.0	≤ 130	134 ± 3	≤ 60
P-RP4	≤ 10.0	≤ 90	113 ± 3	≤ 70
P-RM1	≤ 6.0	≤ 180	80 ± 2	≤ 41
P-RM2	≤ 4.0	≤ 80	88 ± 2	≤ 27
P-RM3	≤ 11.0	≤ 110	112 ± 3	≤ 59

The values obtained from this analysis are within the same range as those reported in Tables 5.1 and 5.2. Again the Na and Cl concentrations show relatively little variability, with the Na concentration of P-LP3 (6030 ± 60 ppm) being the obvious exception. For the other teeth there is no significant difference in Na concentration between left and right counterparts. However, this is not surprising given the limited variability for this element. A much more interesting picture emerges when comparing the values for Cl in each tooth from the left and right side (e.g. P-LM1 with P-RM1). In this case there is a strong correlation ($r^2 = 0.98$) between the concentrations in antimeres. Similarly, a high correlation is found for Zn ($r^2 = 0.94$), but not for Na, Mn and Sr ($r^2 = 0.45$, -0.22, and

0.18, respectively). Chlorine and Zn both show the same trend with a minimal concentration for M1 and increasing concentrations towards the subsequently formed premolars and second and third molars (Fig. 5.2). For Cl the left and right counterparts show more or less the same values, whereas for Zn the left and right teeth show the same trend, but on the right side the Zn concentrations are consistently, and significantly higher ($p < 0.001$, except for P-RM2). Statistically significant differences are also observed for Sr (P-LM2/P-RM2; $p < 0.01$), Na (P-LP3/P-RP3; $p < 0.001$) and Mn (P-LP3/P-RP3, P-LM1/P-RM1 and P-LM2/P-RM2; $p < 0.001$).

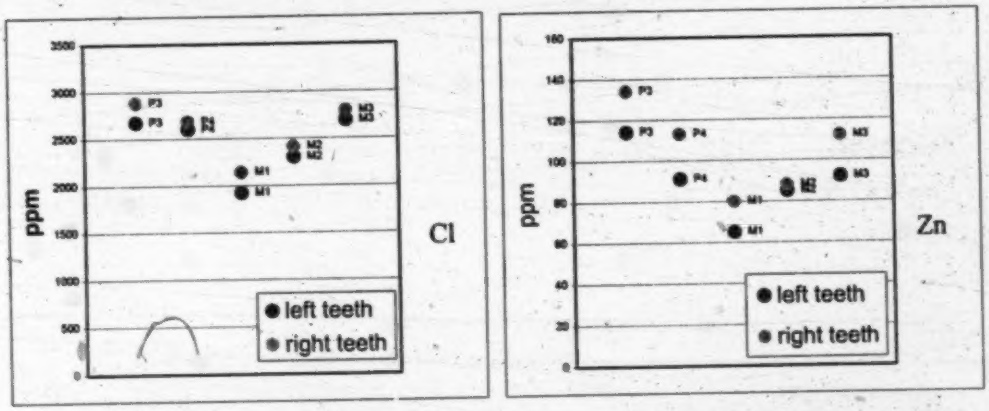

Fig. 5.2: Diagrams showing the relation between concentrations in left and right counterparts for Cl and Zn. Tooth type is shown along the x-axis. These diagrams illustrate the pattern across the different teeth for both elements. The correlations for Cl ($r^2 = 0.98$) and Zn ($r^2 = 0.94$), for concentrations in antimeres, are not directly shown in this figure.

The literature generally reports a lack of significant differences between the left and right counterparts, or between teeth from the upper and lower jaw, within an individual dentition (Curzon *et al.*, 1975; Sachs, 1978). One of the reasons for the present findings could be that trace element levels are variable within tooth crowns and that, consequently, a small sample taken for bulk analysis may not be representative for the whole tooth. The existence of such variation is clearly demonstrated in the data from the microanalytical studies presented below. However, for Zn and Cl, where correlated changes in the left and right counterparts are observed, it is tempting to speculate that tooth-type specific, and possibly diet-related differences have been detected. It will be important to repeat these experiments on multiple dentitions to determine if our finding is more general or an exception.

Microanalysis

Electron probe micro analysis

For our initial microanalytical analyses we used an electron microprobe. In this technique an electron beam is used to generate x-rays in the sample, which can be identified and quantified. The beam can be focused onto small areas of the sample and in this study spot analyses of dental enamel were carried out along the time axis of the crown and from dentine to outer enamel. The following elements were analyzed: Ca, P, F, Na, Mg, Al, Si, S, Cl, K, Fe, Zn, Sr, Ba (see Appendix C for analytical parameters and set-up).

Fluorine, Na, Mg, Cl and K occur at concentrations well above the detection limits. However, Al and Fe were around or below detection limits and could not be reliably measured. Zinc, Sr and Ba were generally present at low concentrations but they occurred at higher concentrations in some areas in some samples. This variability between and within samples indicated the potential value of these elements in palaeodietary studies.

The profiles of elemental concentrations that have been reported in the literature for cross-sections of enamel (e.g., Frostell *et al.*, 1977; Ishiguro *et al.*, 1994) could be reproduced with this analytical technique; Cl and F were found to be highest in the surface layers, decreasing towards the EDJ, and Mg was lowest in the outer layers.

Average Ca/P ratios for the test samples ranged between 1.89-2.01, which is within the range reported in the literature (Wöltgens *et al.*, 1980; Williams & Elliot, 1989). Anomalous values for the major elements (Ca, P) can draw attention to areas of enamel where measurements are likely to be questionable. Causes for such outliers can be cracks in the enamel, irregularities resulting from the polishing procedures, or accumulation of dirt/dust on the sample surface. It was found that the surface damage from the electron beam of the microprobe could be visualized by scanning electron microscopy. SEM video prints showed the individual sampling areas relative to each other and to other markers such as the EDJ. These 'road maps' were found to be essential for the interpretation of the data and were also prepared after laser ablation analysis (see Appendix D and E).

For our pilot studies, intervals of 25 or 5 μm between adjacent points, and beam sizes of 1 or 5 μm were used. Driessens *et al.* (1984), using an electron microprobe, have reported variations in Mg and Na concentrations with a periodicity of 25 μm and 5 μm, respectively. They suggested that the periodicity in Mg concentrations corresponds to striations of Retzius, whereas the periodicity in Na concentrations corresponds to cross striations. As discussed in Chapter 2, these features reflect circadian and circaseptan increments in enamel deposition. These observations illustrate the potential of electron microprobe analysis to detect variation in elemental concentrations at a microscale.

One sample (a modern incisor (T) – see Table 4.1) showed a peculiar pattern in the Ba and Sr distribution in a line perpendicular to the EDJ with a sampling interval and beam diameter of 5 μm. This line started in the dentine close to the EDJ and ran across the enamel to the tooth surface. The inner region of enamel shows quite high concentrations of Sr and especially Ba. Figure 5.3 shows the concentrations of these elements expressed as Ca ratios. Both ratios drop suddenly and significantly about a third of the distance into the enamel, and remain near zero up to the enamel surface. Unfortunately, this finding could not be reproduced during subsequent analyses using the same parameters and analyzing the same area. The sample was also analyzed with a similar microprobe at McGill University by Lang Shi (microprobe technician, Dept. of Earth & Atmospheric Sciences, Univ. of Alberta), but again the analysis failed to yield comparable results (a few hundred ppm Ba and Sr vs. close to 3000 ppm Ba and around 1500 ppm Sr in the first analysis).

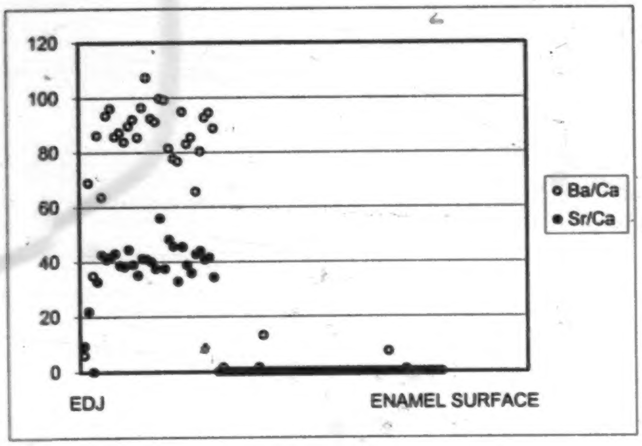

Figure 5.3: The distribution of Sr/Ca and Ba/Ca ratios across the enamel in a modern incisor (T), as determined in a pilot study with the electron microprobe. The line runs from the EDJ to the enamel surface.

In addition to the shift in Ba and Sr concentrations shown in Fig. 5.3, there was a shift in the concentrations of several other trace elements at the same point, whereas the Ca/P ratio remained constant. To determine if this 'boundary' region was a two-dimensional phenomenon rather than a localized feature, it was decided to map the distribution of some trace elements in this particular region of enamel. However, mapping of small areas of the tooth is very time consuming (and thus expensive). Two maps were measured, one with 600 x 600 pixels (pixel size 0.5 µm), and a counting time of 75 msec; the other with 200 x 200 pixels (pixel size 1µm), a counting time of 1 sec, and the beam current doubled to 30 mA. Such maps took ca. 15-20 hours to generate, and yielded very few useful results due to the very low counts per pixel. The mapping procedure could have been improved in several ways, such as using longer counting times while sampling at lower resolution. For example, Lambert *et al.* (1983) produced elemental distribution maps (scan width 400 µm) for bone samples based on counting times of 20 seconds. However, it would be more useful to have distribution maps covering the whole crown instead of such small regions, as we are interested in the elemental distribution along the entire tooth axis. Generating maps for the whole tooth crown was not considered feasible with this technique, given both practical and budgetary constraints.

In general, the electron microprobe can be used for microanalysis of specimens for multiple elements. The technique has been successfully applied in studies of diagenetic changes in bone samples (e.g., Lambert *et al.*, 1983, 1984) and the composition of tooth samples (e.g., Driessens *et al.*, 1984, 1985). However, it is best used for the more abundant elements in the samples, such as Mg, Na, Cl, K and F.

In order to complete an analysis in a reasonable amount of time one is restricted in the number of points to analyze, the number of elements to measure at each point, and the counting time used for each element. In the present study, 14 elements were selected for analysis, and large distances across the enamel were covered at high resolution. Due to these factors, the analytical conditions could not be optimized for each element individually. Longer counting times would likely have improved data quality but time and expense were prohibitive.

Based on preliminary findings, the fact that some trace elements used in the palaeodietary literature (e.g., Cu, V and Mn) cannot be detected in enamel with the electron microprobe, and taking into consideration the budgetary constraints, it was decided that for the final analysis another microanalytical technique was required. The more recently developed technique of laser ablation ICP-MS was considered a better alternative.

Laser Ablation ICP-MS

General discussion of laser ablation analysis and data processing

The permanent and deciduous tooth samples were analyzed using linear tracks running across the enamel parallel to the EDJ from cusp to CEJ (or *vice versa*; longitudinal lines), and tracks starting in the pulp cavity (or inner region of dentine in the absence of pulp) and continuing towards the outside of enamel, crossing the EDJ (cross-sectional lines - Fig. 5.4). Longitudinal lines provide insight into elemental distributions along the tooth axis. The lines running from the inner regions of the dentine to the outer enamel edge provide information about the compositional differences between enamel and dentine, and they can show patterns in trace element distribution within the dentine and enamel regions. Sampling along the lines was a continuous process, with the laser advancing 40 µm/s across the sample.

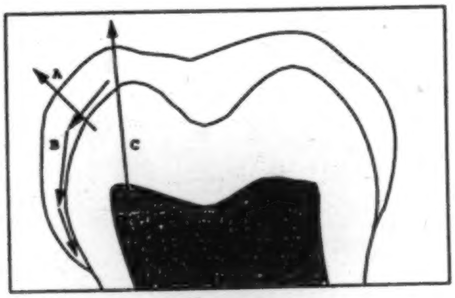

Fig. 5.4: Schematic drawing showing how the longitudinal (B) and cross-sectional (A and C) lines were generally set out on the tooth samples in laser ablation analysis.

The longitudinal lines were chosen in such a way that they would cover most of the developmental period of the crown, crossing the successive incremental lines (striations of Retzius). An attempt was made to keep the laser track close to, and at a relatively constant distance from, the EDJ. However, due to the curvature of the EDJ or other features, such as post-mortem damage, deviations from this ideal were unavoidable. One of the reasons for keeping the beam relatively close to the EDJ is that the thin outer layer of enamel often shows a sharp increase in concentration for several elements (e.g., Zn, Pb). Accordingly, when the beam includes outer enamel areas, concentrations for those elements will increase, giving a false impression of concentration variation in the longitudinal direction. Because the enamel gradually becomes thinner towards the CEJ, trends in elemental concentrations in longitudinal direction will combine with trends in concentrations in the cross-sectional direction when the cervical enamel thickness approaches the width of the beam. With the chosen analytical parameters, this phenomenon posed significant problems in the deciduous teeth, where the enamel of anterior teeth was often as wide as the beam diameter itself.

Composite SEM images were generated for each sample after the analysis. These graphs are included in Appendix D (permanent teeth) and Appendix E (deciduous teeth). With the aid of the composite images the laser ablation data could be correlated with sample features such as tissue boundaries and cracks. Such features were also recorded during data collection. From these notes it became clear that a number of cracks, especially on the deciduous teeth, were induced by the laser during the analysis since they were not visible during data collection but do appear on the SEM images. These cracks tend to follow the course of the laser track, and may be due to the considerable heat generated by the laser.

Scale bars were included on each SEM image. However, measurements of the length of the laser tracks on the images did not in all cases correspond to calculated total distance based on duration of scan and known scan speed. This is due to slight distortions in the SEM images and became especially obvious while making the composite images, when it was sometimes difficult to match corresponding areas on multiple images. Nonetheless, the composite SEM images proved to be an indispensable tool during the analysis and interpretation of the analytical results.

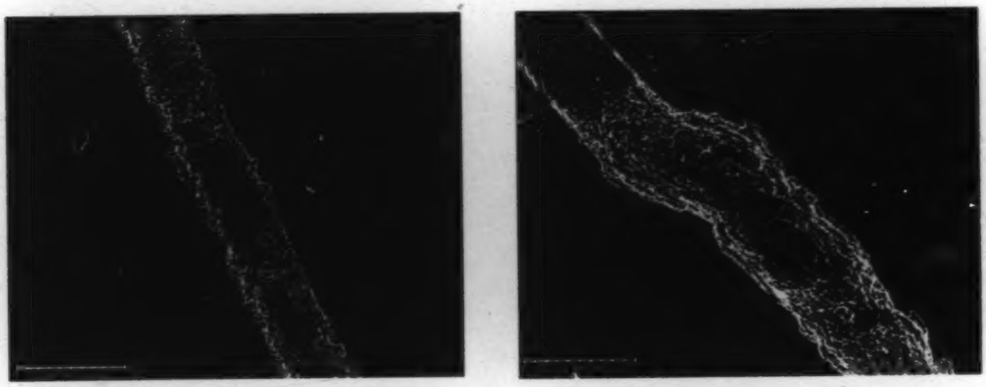

Fig. 5.5: SEM images showing the variability in ablation volume along the laser track on the left permanent third molar (P-LM3). Left: Section along a line in the middle of the enamel including an area where more enamel was removed by the laser; Right: Section of a laser track at the enamel edge of one of the cusps. A layer immediately beneath the outermost enamel layer appears to be more deeply ablated.

An initial visual inspection of scatter plots for all lines showed that both Ca and P were more variable than was expected. This was partly due to the presence of cracks running across the enamel. In addition, SEM imaging of the samples showed that there was some variability in ablation volume along the laser tracks, probably due to local differences in structural characteristics of enamel, for example in relation to microfractures (Fig. 5.5). Obviously, this will affect the counts in an unpredictable way and thus the variability in ablated volume must be compensated for by using one of the major elements as an internal standard. Calcium was selected for this purpose and the effect of this correction is clearly illustrated in Figure 5.6. The raw data are expressed as 'counts per second' of the selected isotopes. As was explained in Chapter 4, the data are only semi-quantitative due to the absence of a suitable standard for enamel. The counts for each isotope have been normalized to the internal standard ^{46}Ca, and in the following discussion all data are expressed as Ca ratios.

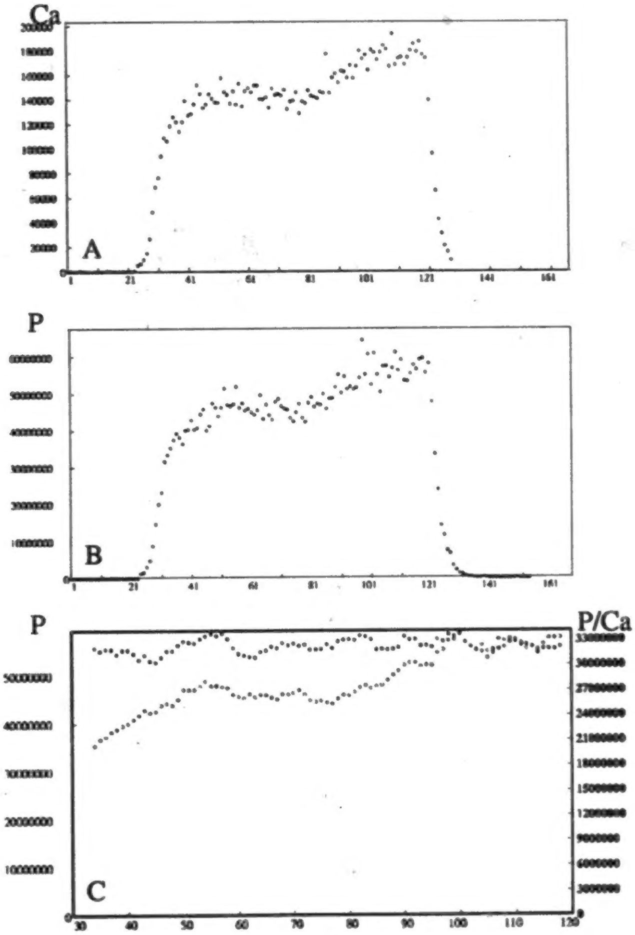

Fig. 5.6: The signal for the Ca and P isotopes during the analysis of line c on the left permanent first molar (P-LM1), running from CEJ to top of crown. A: Ca isotope signal showing the gas blank of ca. 20 seconds (along x-axis), the gradual increase of counts at the beginning and the decay after the laser is turned off at around 120 seconds; B: Idem for P isotope counts; C: Smoothed signal after subtraction of gas blank, removal of outliers and calculation of moving averages with a window of 5. Open circles, primary y axis: P signal; Closed circles, secondary y axis: Ca-calibrated signal for the P isotope (i.e., P/Ca ratio).

Permanent tooth samples

Appendix D shows the SEM composites for all the laser tracks. Some longitudinal lines consist of two or three separate lines. In such cases, the data files were combined into a single data set by joining the appropriate sections of each line.

Trace element patterns along the time axis of the crown

Figures 5.7 to 5.13 show the graphs for each element and its Ca-ratio for one longitudinal line on each tooth analyzed (six left teeth and one right molar). Each line is represented as running from top of crown to CEJ, *i.e.*, from the earliest formed enamel to the last formed enamel. A choice between showing the buccal or lingual lines was mostly based on its representativeness (fewer cracks or other disturbances). Lines not shown here are included in Appendix D.

At first impression, there is considerable variability in the concentrations of the elements across the tooth in the direction of the time-axis. However, some of the peaks (or drops) in counts can be explained by the presence of cracks on the sample surface (see P-LM2 – Fig. 5.11, peaks for Cu, Mn and Zn). This emphasizes the necessity to use SEM images of the samples during the evaluation and interpretation of the data. Because of the 'memory effect', large cracks can affect the counts for a considerable distance along the laser track. If it were not possible to check results against such features on the sample surface, the effects of cracks could easily be interpreted as a biological signal.

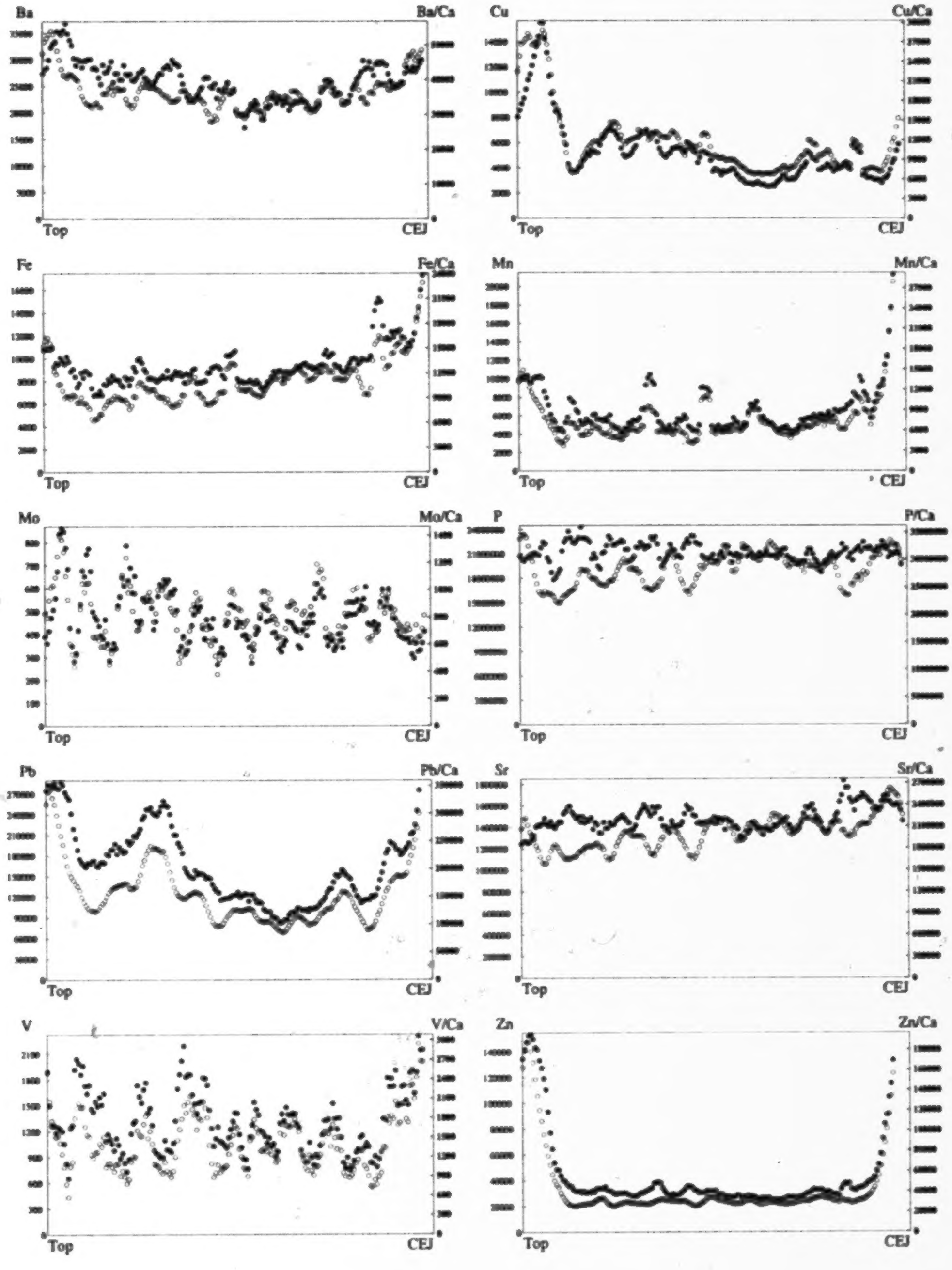

Fig. 5.7: Element and element/Ca ratios for combined lines c and d on the left canine (P-LC) of the maxilla. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

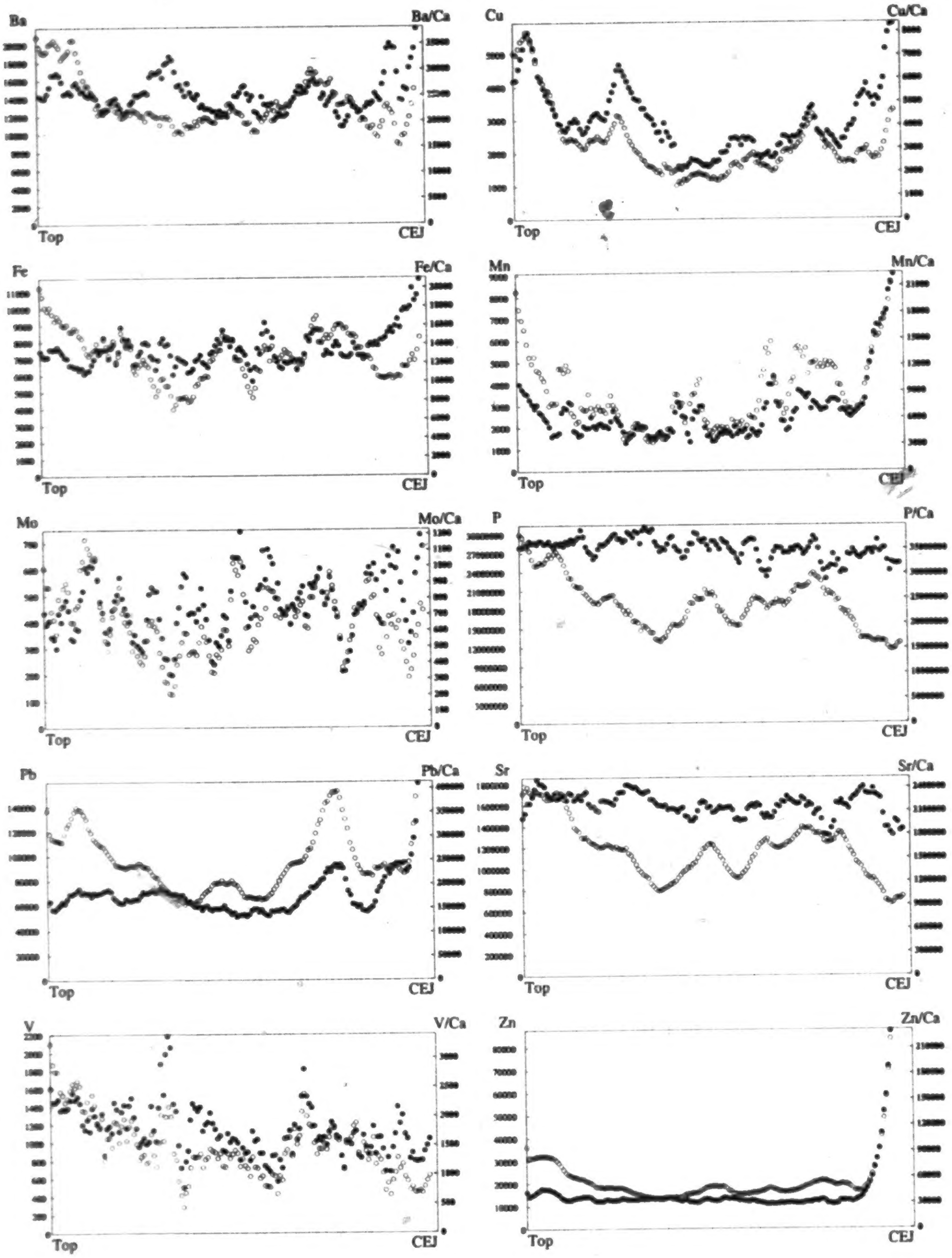

Fig. 5.8: Element and element/Ca ratios for line b on the left first premolar (P-LP3) of the maxilla. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

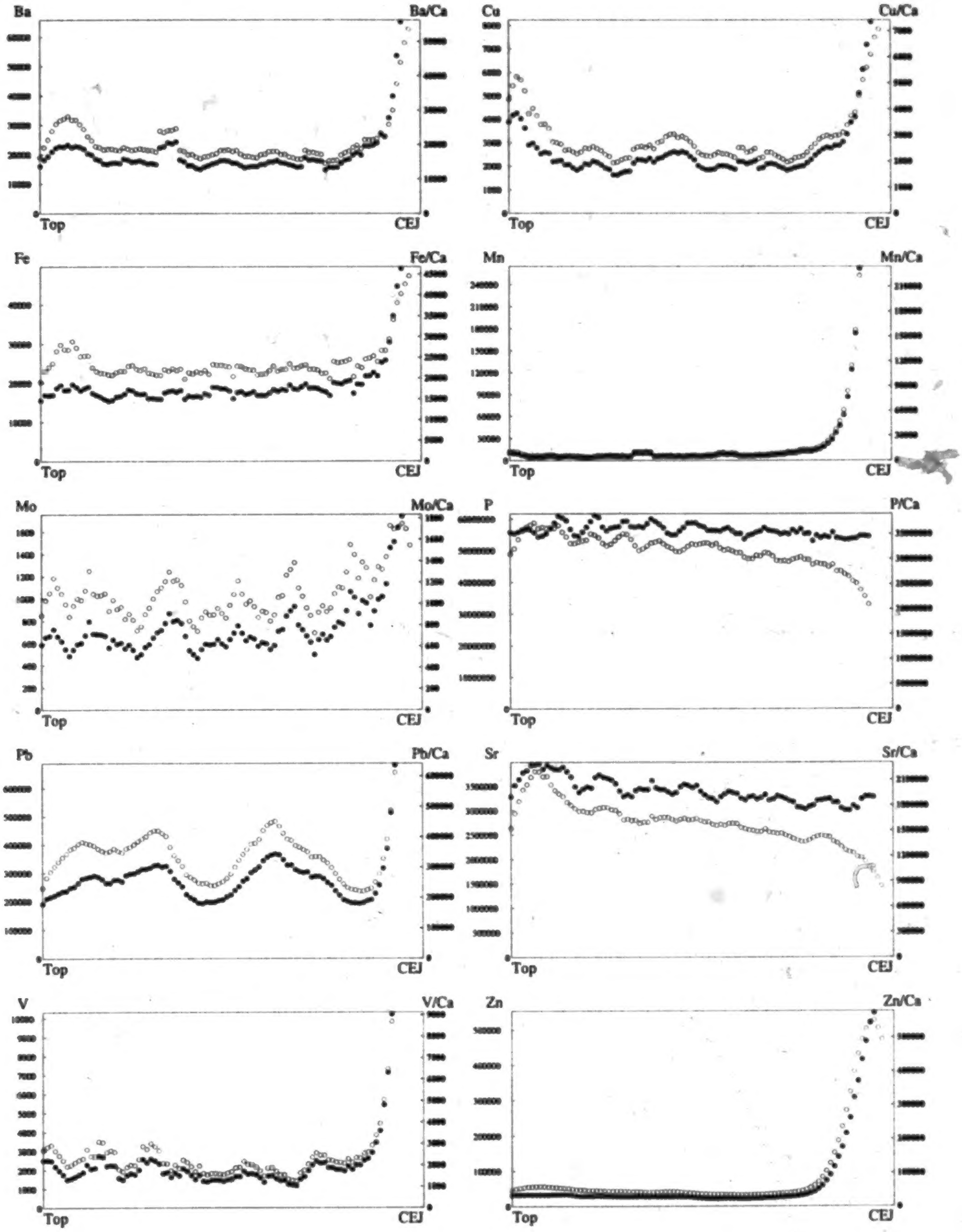

Fig. 5.9: Element and element/Ca ratios for line d on the left second premolar (P-LP4) of the maxilla. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

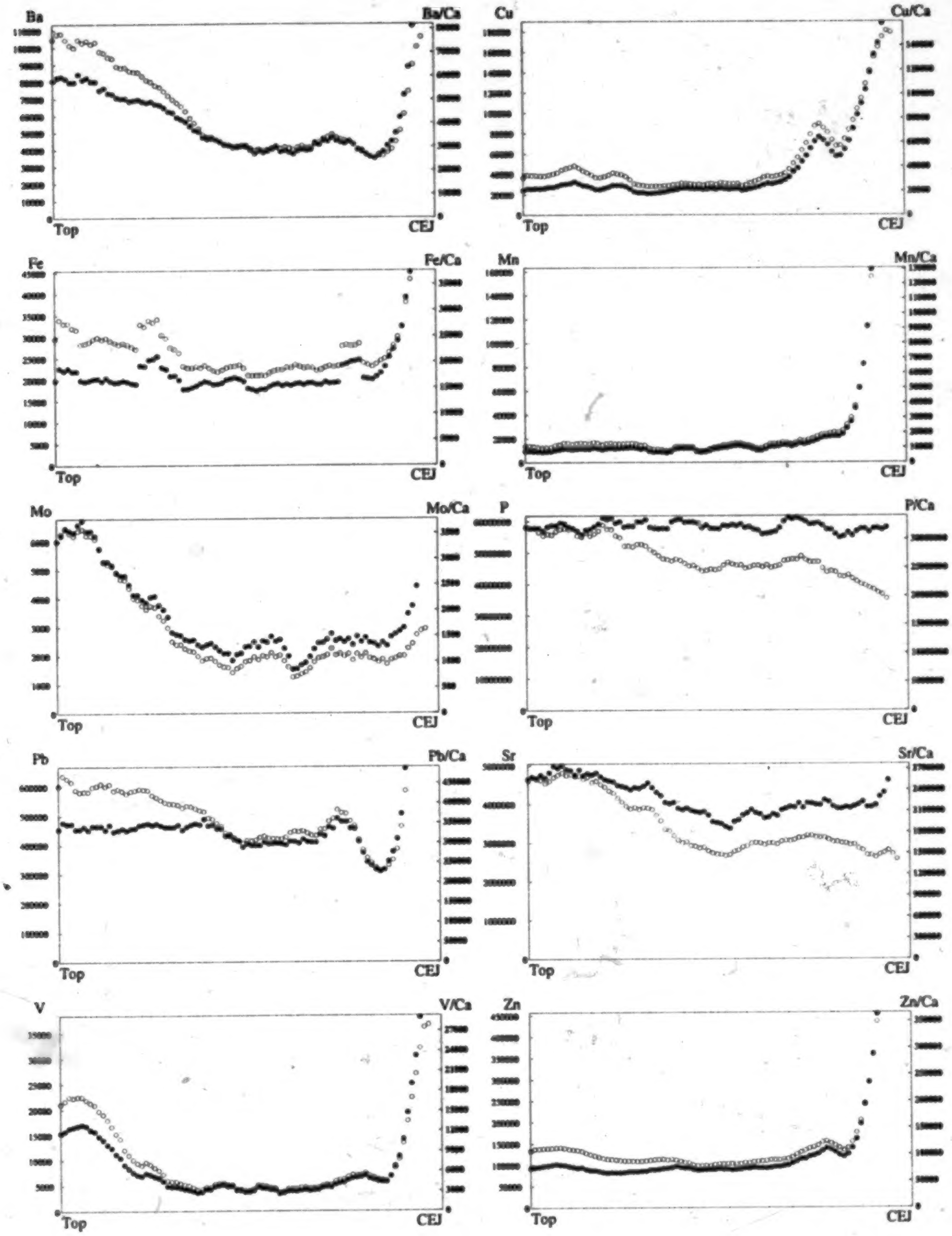

Fig. 5.10: Element and element/Ca ratios for line c on the left first molar (P-LM1) of the maxilla. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

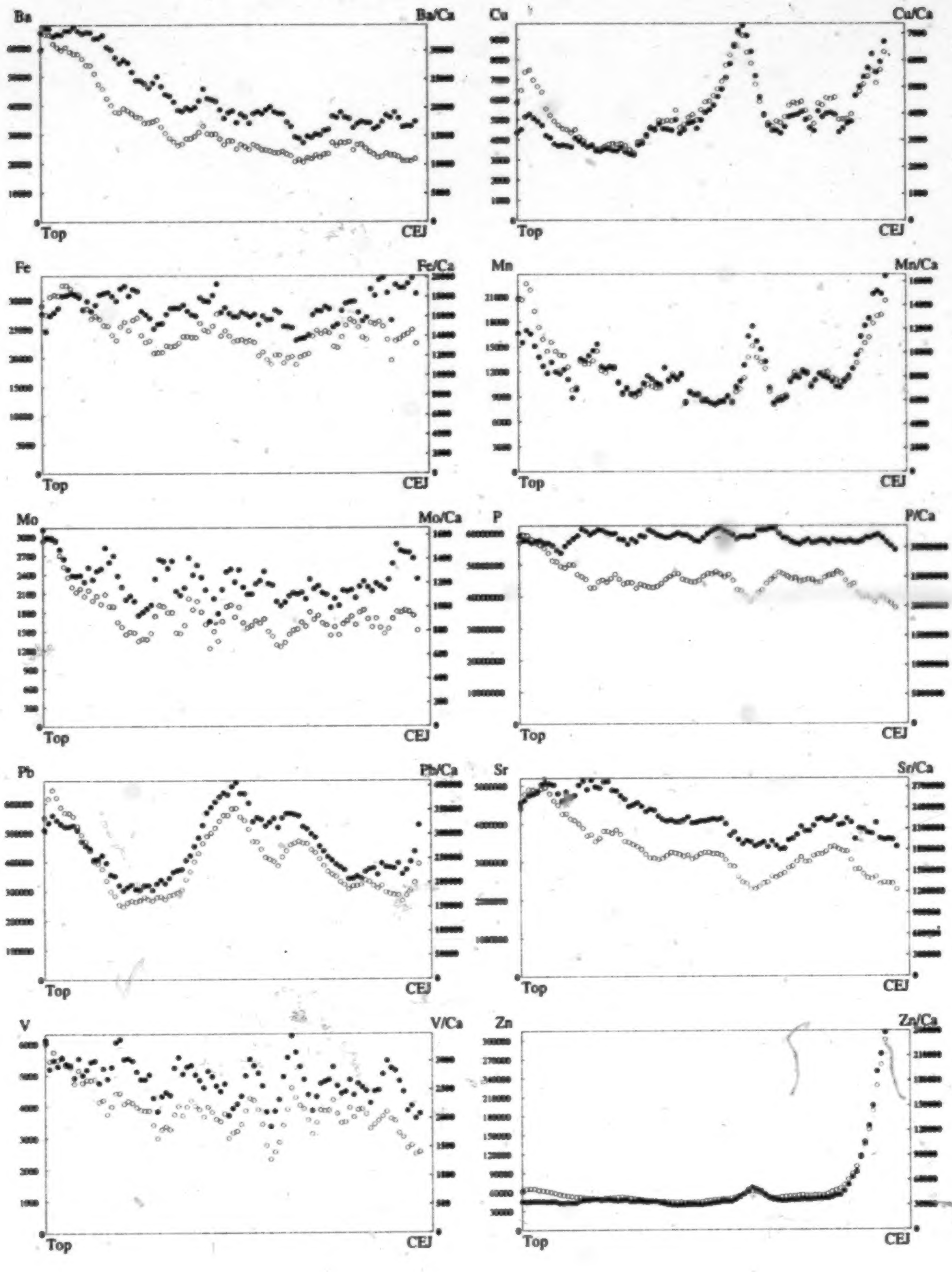

Fig. 5.11: Element and element/Ca ratios for line e on the left second molar (P-LM2) of the maxilla. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

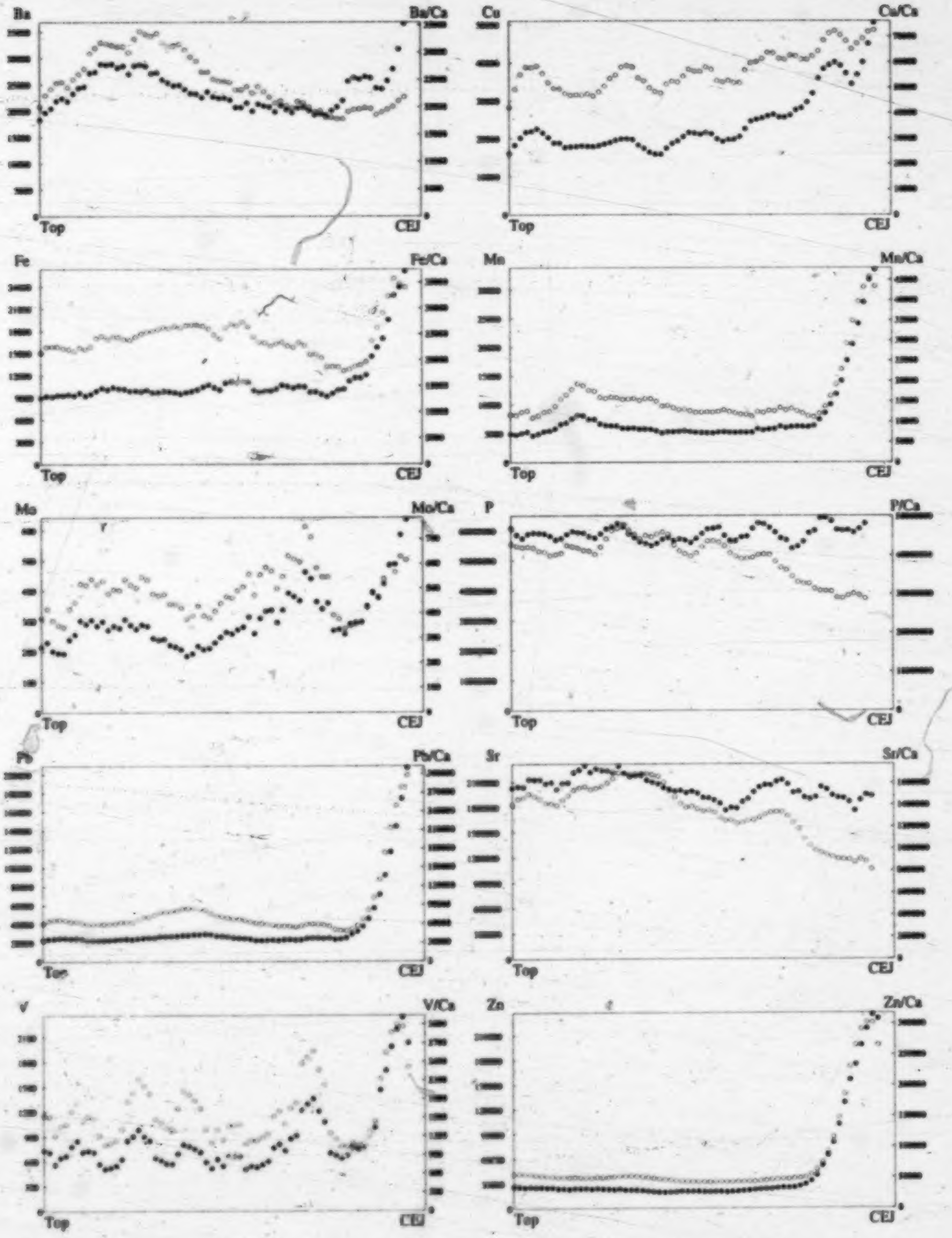

Fig. 5.12: Element and element/Ca ratios for line c on the left third molar (P-LM3) of the maxilla. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

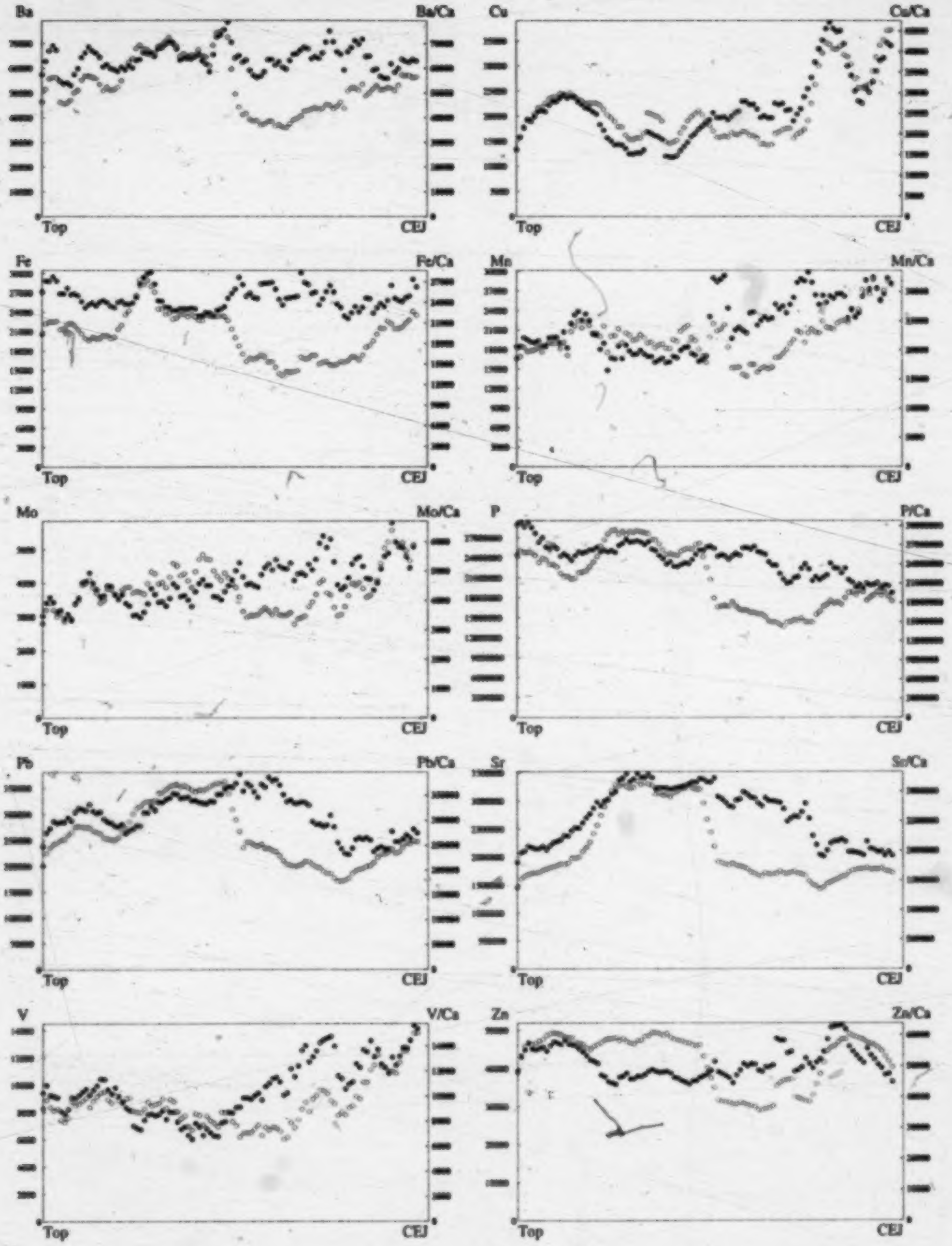

Fig. 5.13: Element and element/Ca ratios for the combined lines a, b and c on the right first molar (P-RM1) of the maxilla. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

In spite of the cracks, visual inspection of SEM composites in conjunction with the data shows that many changes in trace element concentrations along the longitudinal lines must have a different cause. Therefore, the analysis demonstrates that significant intra-tooth variation exists. This proposition is supported by the finding that, in general, the lines on buccal and lingual aspects of the crown show similar trace element distribution patterns (e.g., Pb in P-LP4 lines c and d, Fig. 5.14).

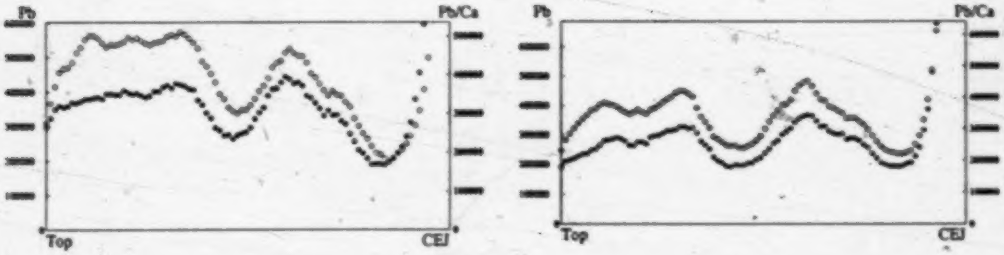

Fig. 5.14: Pb signal for lines c (left; lingual) and d (right; buccal) on the left second premolar (P-LP4), showing the general similarity between the signal from the two opposite aspects of the tooth. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

However, remarkable differences are found for some elements in some lines, such as for example Zn and Sr in lines 'cd' and 'efg' on P-LC (Fig. 5.15), or Cu in P-LP4 lines c and d (Fig. 5.16). Some of these differences could be due to differences in the environment to which the buccal and lingual aspects of a tooth are exposed. For example, moisture and growth of plaque are greatest on the lingual aspect, especially in the cervical region (Purchase & Fergusson, 1986). However, not all differences between lines on the buccal and lingual side of the same tooth occur near the surface, and not all these differences are for the elements with known post-eruptive changes. In the case of Cu in P-LP4 (Fig. 5.16), the concentrations are markedly different on either side of the tooth (reflected in the isotope, as well as the Ca calibrated signal), which is much more difficult to explain.

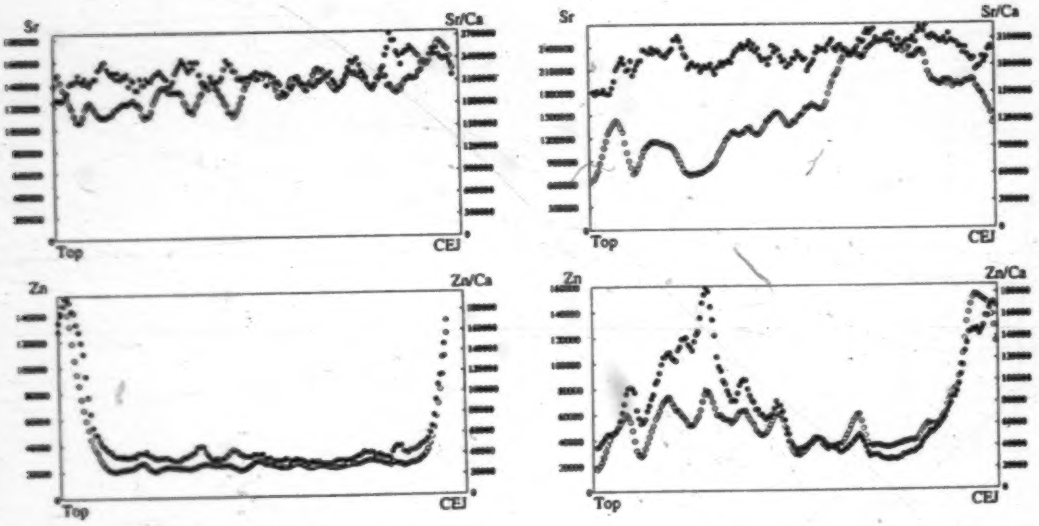

Fig. 5.15: Strontium and Zn measurements along two longitudinal lines in enamel (combined lines c and d (left; buccal) and combined lines e, f and g (right; lingual)) on the left canine (P-LC) showing very different signals on buccal and lingual aspects. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

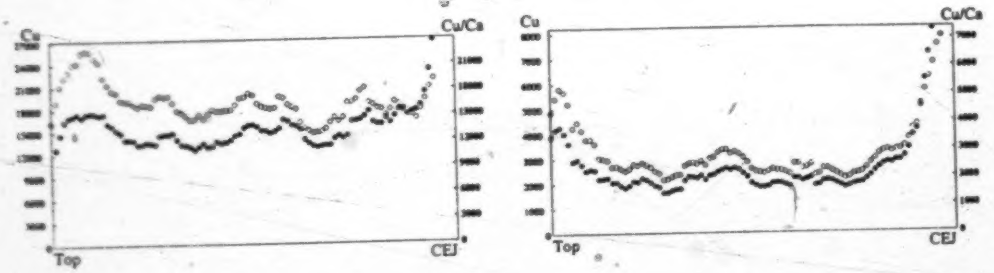

Fig. 5.16: The Cu measurements on lines c (left; lingual) and d (right; buccal) on the left second premolar (P-LP4) show markedly different absolute values on buccal and lingual aspects of the tooth. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

Other possible contributions to some of the differences between buccal and lingual sides are:

- cracks, which may distort the pattern depending on the extent of the damage;
- differences in the actual developmental time covered by the line, depending on the (angle of the) sectioning plane;
- distance of laser track from EDJ and tooth surface (see Chapter 6).

Molybdenum and V concentrations in enamel are generally very low (Mo 1-10 ppm; V < 0.1 ppm) and often below detection limits (Losee *et al.*, 1974a; Curzon & Cutress, 1983). We could detect these elements but the signal to noise ratio was often poor. However, in P-LP4, and most of the molars, these elements showed higher counts than in P-LC and P-LP3, with a concomitantly cleaner signal and sometimes distinct pattern. Copper, Fe, Mn, and Pb are also more variable across the different teeth, without consistent patterns. Zinc appears to be relatively stable along most of the lines.

An interesting observation concerns the distribution of Sr and Ba in the molars. Both elements show higher Ca ratios in the upper part of the crown than in the lower part. This pattern is found in the lines on the buccal as well as the lingual side of the crown (see Appendix D for the lines not shown here). The change in concentration appears to take place more or less halfway down the crown in all the molars. Since the three molars develop during quite different periods, this common pattern suggests that an explanation other than dietary intake is required to account for the observations. The fact that P-LM3, which had not yet erupted, shows the same pattern as P-LM1 and P-LM2, indicates that this particular distribution of Sr and Ba is established during development, and does not arise as a result of post-eruptive processes. In addition, the point where the shift takes place is situated away from the enamel surface, where post-eruptive uptake takes place. It has also been suggested that Sr is mostly incorporated into enamel during maturation, and shows little post-eruptive uptake (Steadman *et al.*, 1958). Since the chemical behaviour of Ba is very similar to that of Sr, the same may be true for this element. The underlying cause of the distributions of Ba and Sr is not immediately clear, but may be related to the fact that these elements are less abundant near the enamel surface, as discussed below.

In P-LP4, Pb shows some peaks in the longitudinal lines (see Fig. 5.9). Lead peaks are also found in P-LC, P-LP3, P-LM2, and to a much lesser extent in P-LM1, P-LM3 and P-RM1. The cross-sectional lines, which go across the dentine and enamel (see below - Fig. 5.18; for the separate lines see Appendix D), show that Pb peaks are also found in the dentine. The lines for P-LP3 and P-LP4 (see Appendix D) have a distinct shape, consisting of three sequential peaks, with the middle one being the highest. This repetition of a pattern in several lines on the same tooth (esp. the parallel cross-sectional lines on P-LP3) and across multiple teeth within this dentition, suggest that this reflects a series of events, resulting in temporarily higher Pb incorporation, during the life of this individual. Since there is no background information for this individual, a possible reason for the increased Pb incorporation cannot be given. However, this feature could provide insights into how the chemical composition of the different teeth correlates with developmental time. It would be interesting to have a 2-D map of P-LP3, to study the distribution of the Pb lines across the tooth. A histological analysis of several of these teeth should be carried out to determine if the Pb peaks in the different teeth occur at about the same developmental age.

Behaviour of elements from inner to outer enamel

The behaviour of the elements across the thickness of enamel could be determined from the cross-sectional lines, as well as from several parallel longitudinal lines that were analyzed on some of the samples. Figure 5.17 summarizes the observed (generalized) trends for each of the element/Ca ratios.

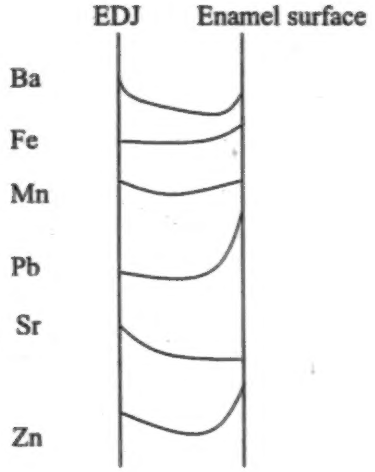

Fig. 5.17: Generalized trends for the Ca ratios of some of the trace elements as observed in the permanent maxillary teeth. Copper, Mo and V showed no systematic trends and are therefore not included in the graph.

Summary graphs of laser tracks moving from inner dentine to outer enamel edge on each tooth are shown in Figure 5.18 (cross-sectional lines for each tooth separately are included in Appendix D). To produce these graphs, the datasets were first processed as described in Chapter 4 (p. 115). Subsequently, the data points were scaled so that the distance between the start of the line in the dentine ('Pulp' in the Figure) and EDJ, and between EDJ and outer enamel surface ('Edge') are each represented by 40 points. Although this slightly distorts the picture – the distance across enamel is in reality shorter than the distance across the dentine – it allows one to compare concentration profiles along laser tracks of different lengths.

In Figure 5.18 the plots for the Ba/Ca and Sr/Ca ratios show that both ratios gradually decrease from EDJ to outer enamel. Values near the EDJ are almost twice as high as those in outer enamel. In contrast, Frostell *et al.* (1977) found a nearly homogenous distribution for Sr, except for a decrease in the outermost enamel. The pattern of higher Sr/Ca and Ba/Ca ratios near the EDJ found in our study is actually comparable to the somewhat mysterious electron microprobe results for the modern incisor described on p. 125 and shown in Fig. 5.3.

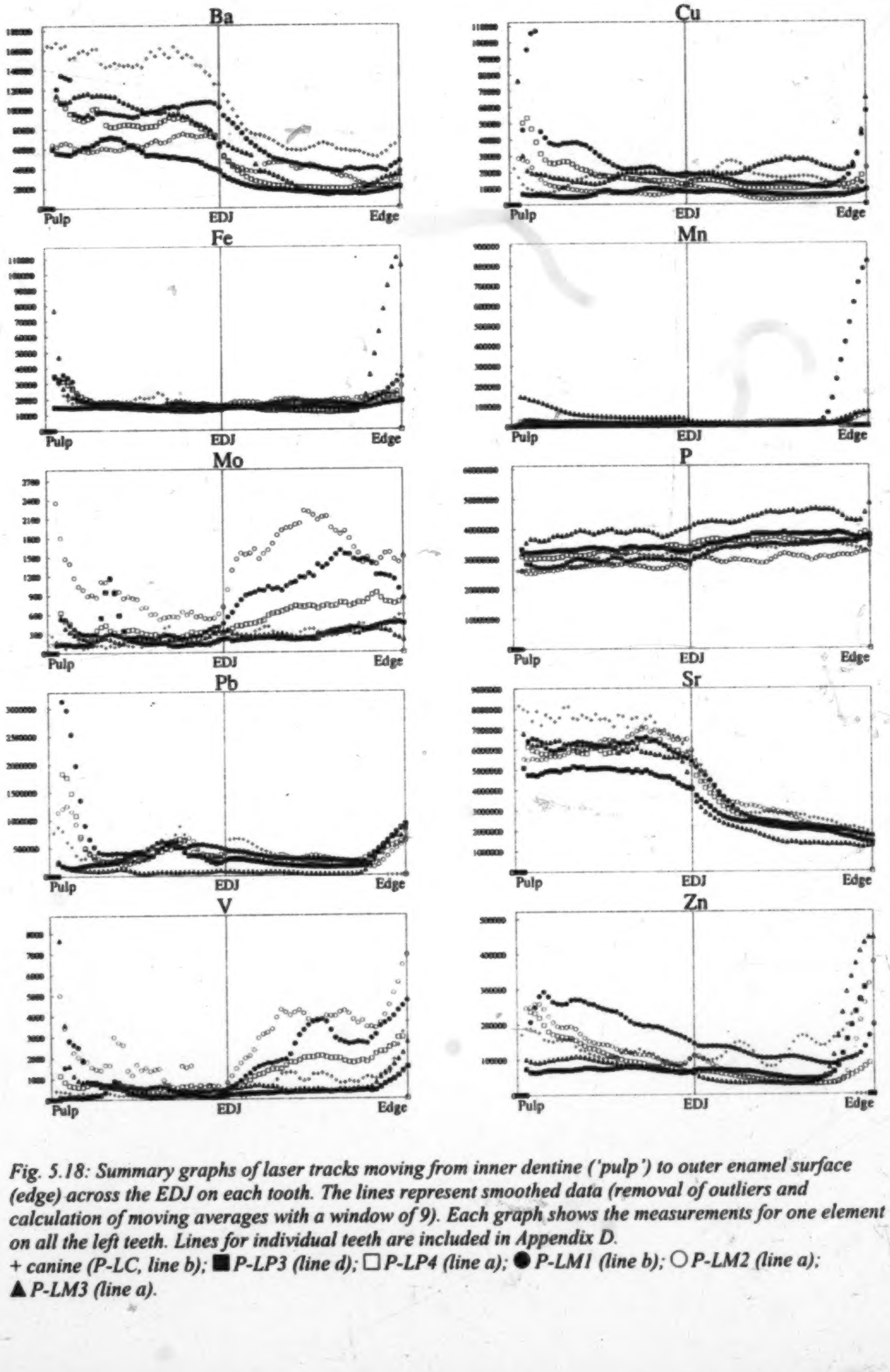

Fig. 5.18: Summary graphs of laser tracks moving from inner dentine ('pulp') to outer enamel surface (edge) across the EDJ on each tooth. The lines represent smoothed data (removal of outliers and calculation of moving averages with a window of 9). Each graph shows the measurements for one element on all the left teeth. Lines for individual teeth are included in Appendix D.
+ canine (P-LC, line b); ■ P-LP3 (line d); □ P-LP4 (line a); ● P-LM1 (line b); ○ P-LM2 (line a); ▲ P-LM3 (line a).

The gradient measured with LA-ICP-MS is not as abrupt, but that may be due to the memory effect that occurs during the laser analysis. To some extent this distribution may contribute to the finding that along the longitudinal lines of the molars the ratios for Sr and Ba are decreasing towards the CEJ where relatively more of the outer enamel is sampled.

Molybdenum and V do not show a clear trend but their low counts may prevent the detection of weak trends. In contrast, Pb and Zn show a distinct peak at the enamel surface, which is also reported in the literature (e.g., Purchase & Fergusson, 1986). Similarly, Fe, Mn and Cu display a surface peak, albeit of varying intensity (Cu, Fe: Purchase & Fergusson, 1986). Most surface effects are thought to be due to post-eruptive or (for archaeological samples) diagenetic changes. Surface elemental concentrations are therefore more useful as environmental indicators than as indicators of diet.

Differences between teeth of the same individual (archaeological specimen from the Netherlands)

The gradual decrease or increase for some elements, and the more marked variations in concentrations for other elements along the longitudinal lines (Figs. 5.7-5.13) indicate that the data are not normally distributed. This makes it difficult to provide a summary of the observations and to compare the concentrations of the elements in the different teeth. The calculation of average Ca ratios for the trace elements for each tooth will not adequately capture the differences and similarities among the different teeth of this dentition. Therefore, box plots were chosen as a way of graphically representing a measure of central tendency as well as the spread in the data (Fig. 5.19a). Because the concentrations for some elements change about halfway down the crown, the datasets were also split up into two subsets ('upper crown' and 'lower crown'), which were analyzed separately (Fig. 5.19b).

The box plots clearly show that, in comparison with the other teeth, P-LM3 has distinctly higher P/Ca ratios, which may indicate that its mineralization is not complete. The other striking difference is the extremely low Pb/Ca ratio. This observation agrees with the fact that Pb accumulates in the enamel surface layers throughout life, reflecting

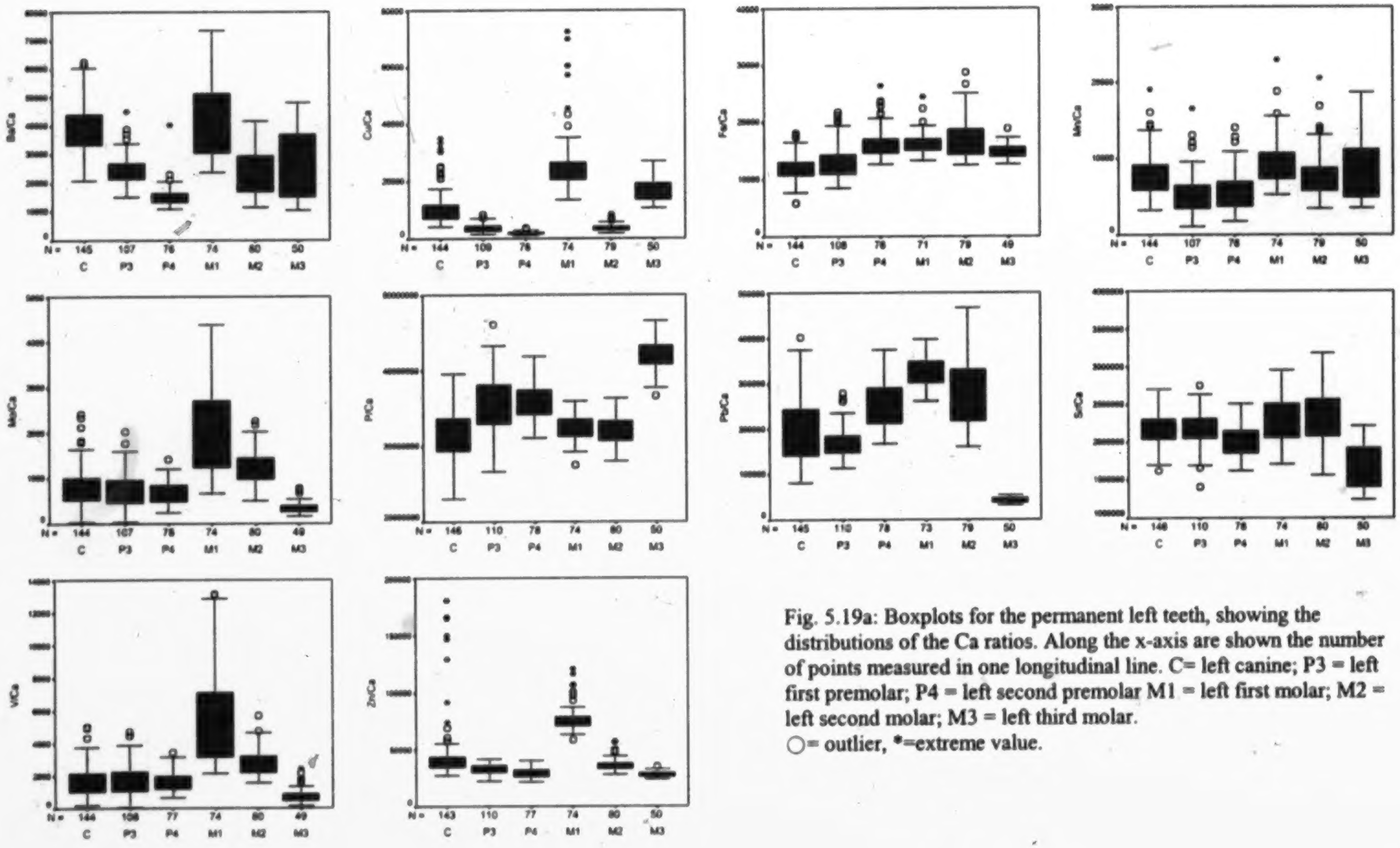

Fig. 5.19a: Boxplots for the permanent left teeth, showing the distributions of the Ca ratios. Along the x-axis are shown the number of points measured in one longitudinal line. C= left canine; P3 = left first premolar; P4 = left second premolar M1 = left first molar; M2 = left second molar; M3 = left third molar.

○= outlier, *=extreme value.

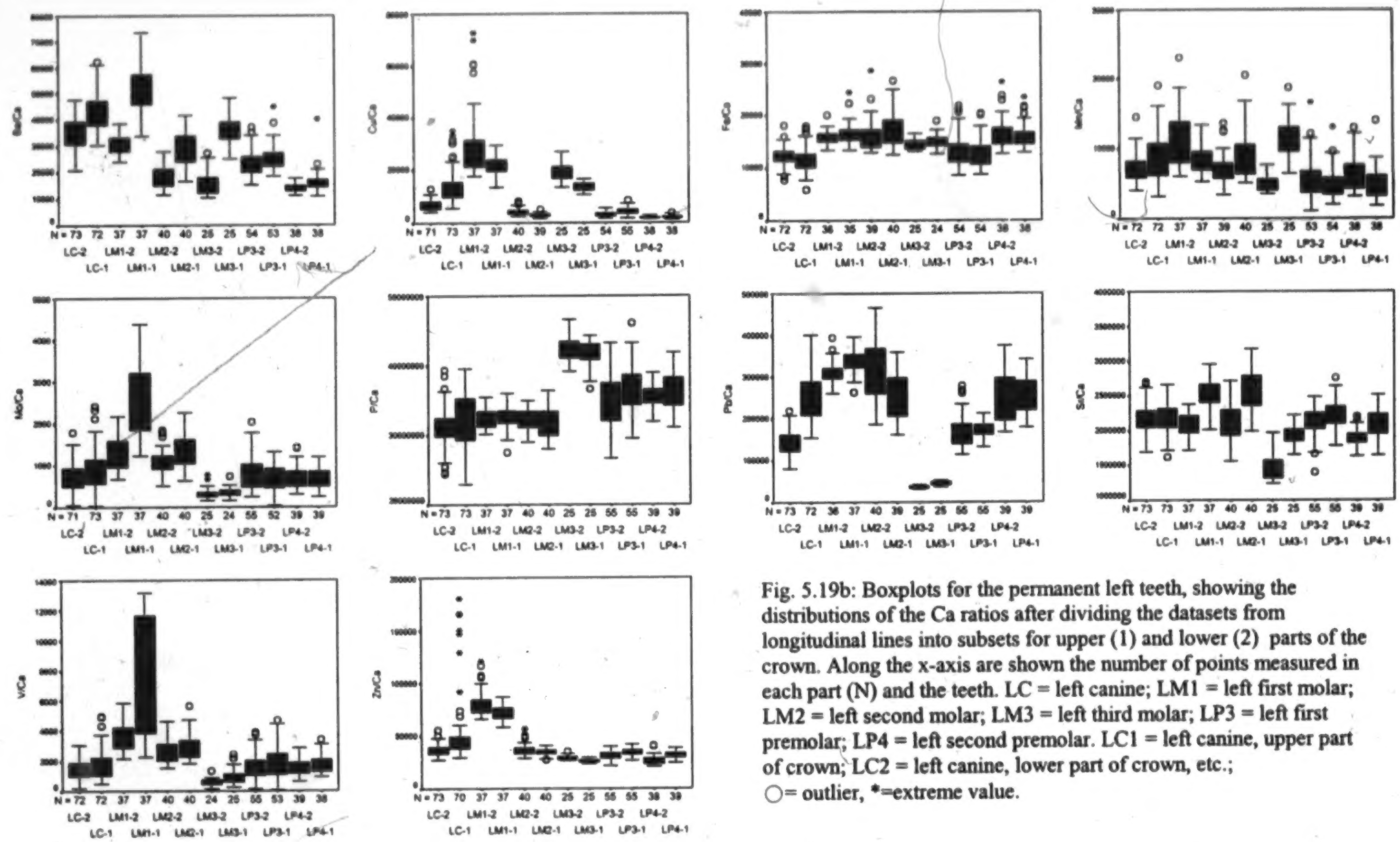

Fig. 5.19b: Boxplots for the permanent left teeth, showing the distributions of the Ca ratios after dividing the datasets from longitudinal lines into subsets for upper (1) and lower (2) parts of the crown. Along the x-axis are shown the number of points measured in each part (N) and the teeth. LC = left canine; LM1 = left first molar; LM2 = left second molar; LM3 = left third molar; LP3 = left first premolar; LP4 = left second premolar. LC1 = left canine, upper part of crown; LC2 = left canine, lower part of crown, etc.;
○= outlier, *=extreme value.

environmental exposure (Brudevold & Steadman, 1956). It is interesting to note that, in spite of its unerupted state, there is already a clear Pb peak in the outer enamel layer (Fig. 5.18). Accordingly, this Pb accumulation at the surface already starts before eruption, as was also reported by Brudevold & Steadman (1956). There does not appear to be a correlation between Pb concentrations in the different teeth and time since eruption, as was found by Purchase & Fergusson (1986).

There also appears to be a difference in Pb/Ca ratios in the upper and lower half of the P-LM3 crown, with the lower, less matured, portion of the crown having the slightly lower Pb/Ca ratio. This effect is more pronounced for the Ca ratios of Sr and Ba. It is tempting to speculate that this distribution pattern indicates that these elements accumulate during maturation, as reported by Olsen & Jonsen (1979). However, the pattern of lower Sr/Ca ratios in the lower half of the crown is found for all three molars, which argues that incomplete mineralization can only partly account for the observed pattern. For P-LM1, this pattern is contrary to the general expectation based on the assumption that the upper part of the first molar crown is formed during the period of breastfeeding (usually characterized by low Sr intake) and that the later formed parts of the crown correspond to a post-weaning period (higher Sr intake). Both Mo and V show the highest value and greatest variance in the upper half of P-LM1 (Fig. 5.19b). A shift in their concentrations occurs around mid-crown, as is the case for Sr and Ba, and this is clearly shown in the plots illustrated above in Fig. 5.10.

The box plots also allow us to observe the increased levels of Zn and, to a lesser extent, Cu in P-LM1. These two transition metals are similar in their chemical behaviour. Both can bind to proteins, and – in the palaeodietary literature – are considered markers for meat consumption. No background information is available for this individual and dietary interpretations are not possible without contextual information and an understanding of the relation between dietary intake and enamel composition.

Curzon *et al.* (1971) reported that surface enamel of unerupted and newly erupted premolars, unlike teeth of older individuals, contained no Mo. This seems to imply that this element is mainly taken up post-eruptively. However, others have reported that Mo uptake takes place during the maturation stage in rat molars (Bawden & Hammarström, 1976), which is in agreement with the measurement of Mo in the unerupted P-LM3 in our study. Most likely, the findings of Curzon *et al.* (1971) can be explained by the generally low Mo concentrations in enamel, which makes it difficult to measure this element accurately. Our finding of higher levels of Mo in the P-LM1, which - of the teeth analyzed - is the first tooth to erupt, suggests that this element is indeed acquired post-eruptively as well.

Comparison of laser ablation data and NAA data

Of the elements selected for laser ablation analysis, only Sr, Zn, Mn and Fe were detected with instrumental NAA, with Fe being around detection limits. Phosphorus can not be detected using this technique. The Pb-radionuclide produced in the reactor has a very short half-life (796 ms) and can therefore not be counted. The Cu-radionuclides, ^{64}Cu and ^{66}Cu, are generated at very low rates. Consequently, the signal for this element is easily overshadowed by other more readily activated elements. Vanadium occurs at very low concentrations in enamel and was below detection limits. The detection of Mo is complicated by several factors. The radionuclide ^{99}Mo, which does not activate very well, produces its most intense gamma-ray at 140.5 keV, and its detection is hindered by ^{24}Na and ^{32}P Bremsstrahlung. In addition, Mo occurs at very low concentrations in enamel. Finally, the most sensitive radioisotope of Ba (^{131}Ba) is difficult to detect because its parent-isotope ^{130}Ba has an isotopic abundance of only 0.106% (J. Duke, *pers. comm.*).

A conflicting observation was made for the determination of Zn levels by the two techniques. With NAA, P-LM1 from the archaeological maxilla has a lower Zn concentration than all other teeth from the same quadrant (Table 5.4). In contrast, LA-ICP-MS finds that P-LM1 has a clearly higher Zn concentration than all the other teeth (Fig. 5.19a). The discrepancy between the results from the two techniques could be due to the difference in sampling. With NAA, sections of the crown that contained the full length of the crown on both the buccal and lingual sides were analyzed. With laser ablation analysis, the lines include mostly the inner part of the enamel in the upper half, and relatively more of the outer enamel in the lower half. Another important point to keep in mind is that NAA yields absolute concentrations in ppm, whereas the laser ablation results in our study are expressed as Ca ratios. Therefore, at least theoretically, the increase in Zn/Ca ratio found by LA-ICP-MS could result from a lower concentration of

Ca, rather than a higher concentration of Zn, in this tooth. Although this would be surprising, it is worth noting that for P-LM1 all elements show – to varying degrees – higher values, consistent with a depression of calcification. The only exception is P, but since phosphorus uptake into apatite is directly coupled to calcium incorporation this is as expected.

Deciduous tooth samples

The deciduous tooth samples were selected so that for each individual every tooth type was included. However, for individual A the canines were not available, and for individual C some of the incisors could not be used due to extreme wear. Due to factors such as wear and fracturing, the teeth that were selected did not all come from the same quadrant. The NAA results have shown that there can in fact be differences between sections of left and right teeth, and this should be kept in mind during evaluation of the data.

[The results discussed below are concerned mainly with the tooth samples from individuals A, B, and C]

For the laser ablation analysis of the deciduous teeth, the same parameters were chosen as for the permanent teeth. Because the enamel of these teeth is much thinner than for permanent teeth, the beam width of between 150-200 μm was a problem for some of the samples. For the anterior teeth, which have thin enamel, the beam was sometimes as wide as the enamel. Consequently, the data represent contributions of both the inner and outer enamel layers and in some cases the beam even crossed over into dentine or epoxy. These particular data sets therefore provide a mixture of signals, and are difficult to interpret.

Behaviour of elements from inner to outer enamel

To determine the distribution patterns of trace elements across the thickness of enamel in all individuals, the subset of enamel data points from cross-sectional lines (molars only) were analyzed. Figure 5.20 shows the generalized behaviour of the elements from inner to outer enamel. Data from parallel longitudinal lines that had been analyzed on several molars provided additional information, as well as a series of 2-D elemental distribution maps, collected from the second molar of individual C (C-Lm2; Fig. 5.21).

A comparison of two sets of parallel lines on the second molar of individual A (A-Rm2; Fig. 5.22 e-h) showed that, in general, the patterns in trace element distribution are very similar in outer and inner enamel. Where patterns deviate this is usually due to the outer line approaching the tooth surface, where elements such as Zn and Pb are found in higher concentrations.

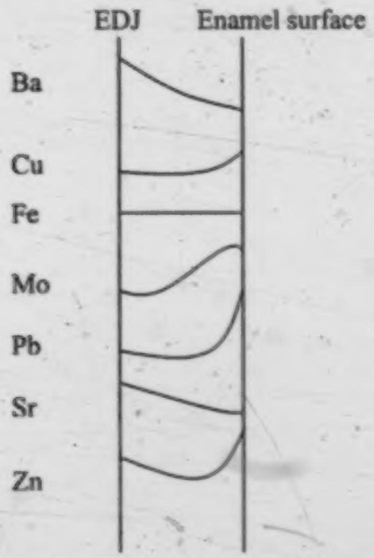

Fig. 5.20: Generalized trends for the Ca ratios of the trace elements from inner to outer enamel in the deciduous molars of individuals A, B and C. Manganese and V did not show any specific trend and are therefore not depicted.

Elemental distribution across the whole crown: 2-D maps

More information about the distribution patterns of the elements in enamel was obtained in the form of 2-D distribution maps for each element measured on the second deciduous molar of individual C (C-Lm2; Figures 5.21).

In general, Ba and Sr are lower in the enamel than in the dentine. Within enamel, these elements show a gradual increase towards the EDJ. All the other elements show higher ratios in the outer enamel, as shown by the dark blue and purple colours, with a gradual decrease towards the EDJ. However, these distribution maps clearly show that there can be localized differences, or a more patchy distribution (e.g., Mn, Zn). The implication is that the choice of location for the longitudinal or cross-sectional lines will to a certain degree determine our idea of how an element is distributed within the different tissues and how it behaves across tissue boundaries. For this particular molar, for example, two lines set out from pulp to outer enamel through the two major cusps will give a very different idea of the distribution of Pb/Ca ratios across the tooth.

Zinc shows a somewhat peculiar distribution pattern in the middle cusp. The outer layers with high Zn/Ca ratios (dark blue and purple) are discontinuous across the cusp, which creates the suggestion of a worn enamel surface. However, this is not in agreement with the image on the SEM composite. Again, two lines set out from the pulp cavity through both of the major cusps would lead to very different conclusions about the distribution of Zn/Ca ratios.

The generalized trends across the thickness of enamel, as shown in Fig. 5.20, are based on a number of lines, which cover only a small section of enamel. The observed trends from inner to outer enamel along cross-sectional lines are likewise affected by the sometimes patchy distribution. The colour map for Fe clearly shows that this element is more variable across the width of enamel than is suggested by Fig. 5.20.

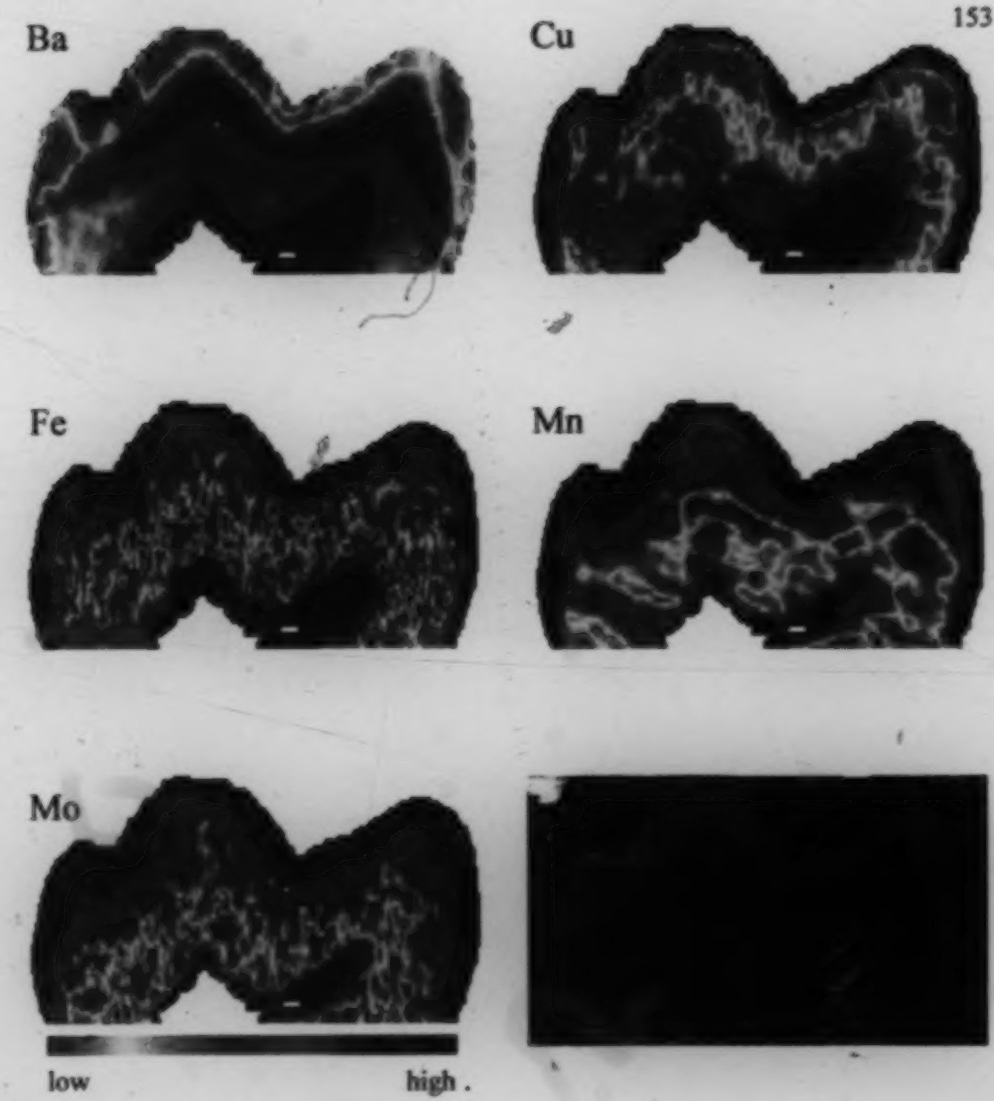

Fig. 5.21: The two-dimensional distribution of element/Ca ratios in C-Lm2, the deciduous second molar of individual C. The tooth was scanned from the upper left to the lower right, as shown on the SEM composite image (width of tooth at midcrown is c. 11 mm.) The colour maps are based on centered 5x5 averages for measurement points on the tooth only (epoxy was set to zero). Colours represent low (red) to high (purple) Ca ratios as indicated by the scale below the map for the Mo/Ca ratios and Zn/Ca ratios.

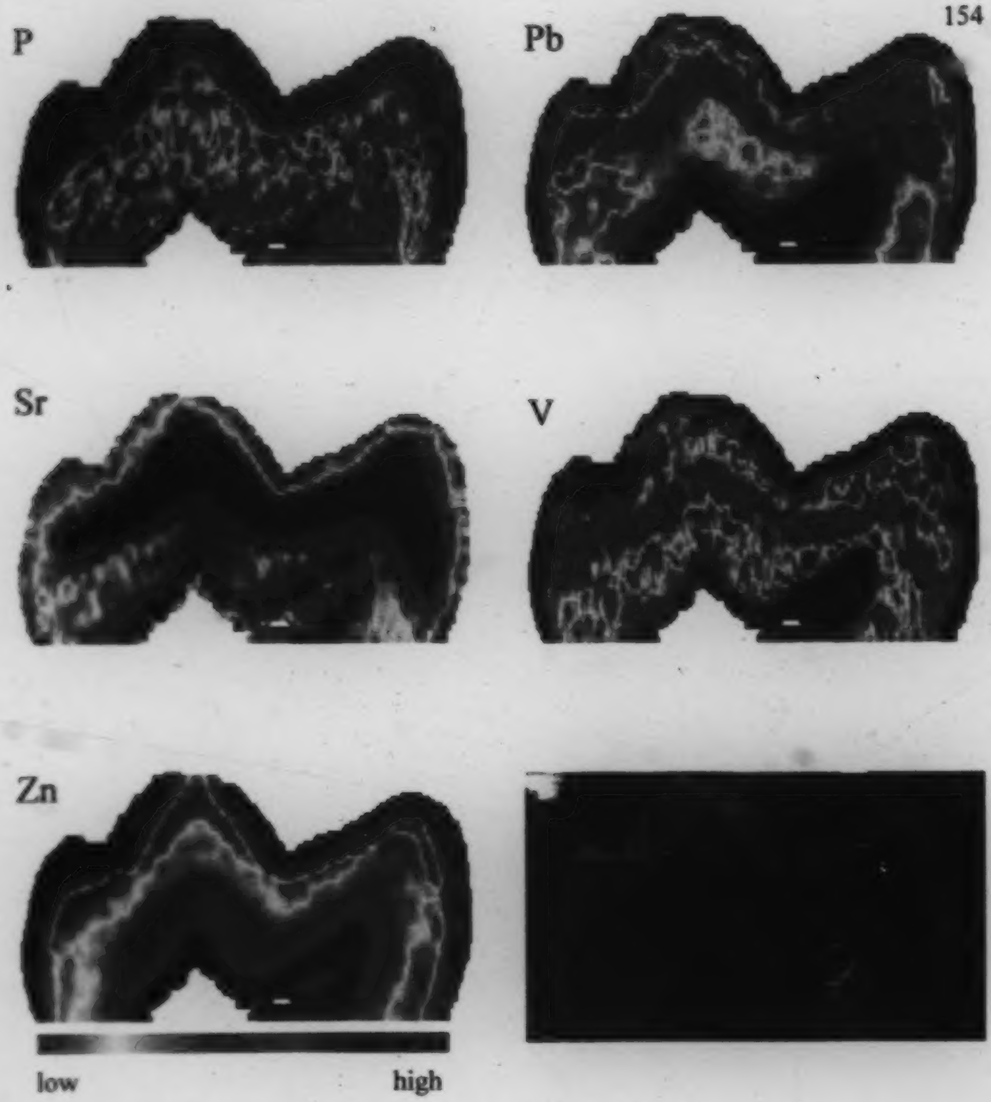

Fig. 5.21 (continued).

Trace element patterns along the time axis of the crown

The plots showing the longitudinal lines (Figs. 5.22 – 5.24) illustrate the variability in elemental concentrations along the length of the crown. As was the case for the permanent tooth samples, some of this variability is due to cracks and other surface features, which obscure the general pattern. For example, the peak for several elements in the middle of line 1 on A-Li1 (Fig. 5.22 a) coincides with an area where the beam just touched the EDJ. Likewise, the peaks in the graphs for line 5 on A-Rm2 (Fig. 5.22 h) appears to be in the region that shows up on the SEM as a more deeply ablated section of the track (Appendix E). In addition, for an evaluation of the patterns within a single longitudinal line, the behaviour of the elements in a cross section of enamel must be considered (see above). For example, the gradual decrease in the Ba concentration along lines 4 and 5 (A-Rm2) is most likely due to the lower concentrations of Ba in the outer enamel regions, since more of the outer enamel is sampled towards the end of these lines.

However, many of the trends that can be observed in these longitudinal lines do not appear to be associated with any of these artefacts, and therefore seem to represent a real signal that is clearly above the level of noise. An example is the decreasing trend for Mn on B-Ri1(Fig. 5.23 a). A similar trend for Mn is seen in the longitudinal line on tooth C-Ri1 (Fig. 5.24 a, b). From these observations we can conclude that LA-ICP-MS is capable of detecting intra-tooth variation in trace element concentrations. A re-analysis of the samples with optimized analytical parameters (see Chapter 6) will be necessary to reduce or eliminate some of the complications in the current data. Because of these complexities, it is premature to draw any firm conclusions from the data on intra-tooth variation in terms of possible underlying dietary causes.

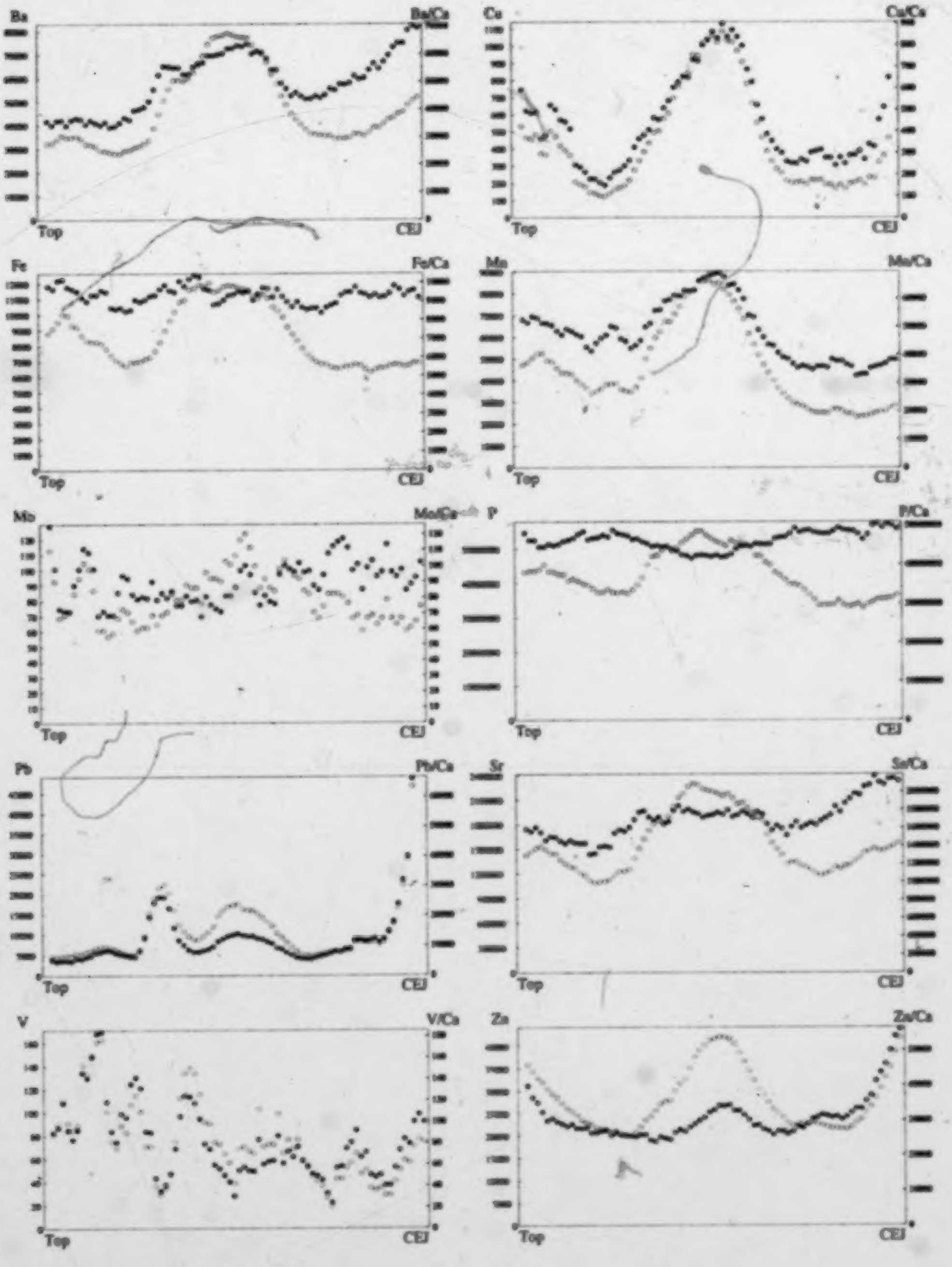

Fig. 5.22a: INDIVIDUAL A: Element and element/Ca ratios for line 1 on the left central incisor (A-Li1). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

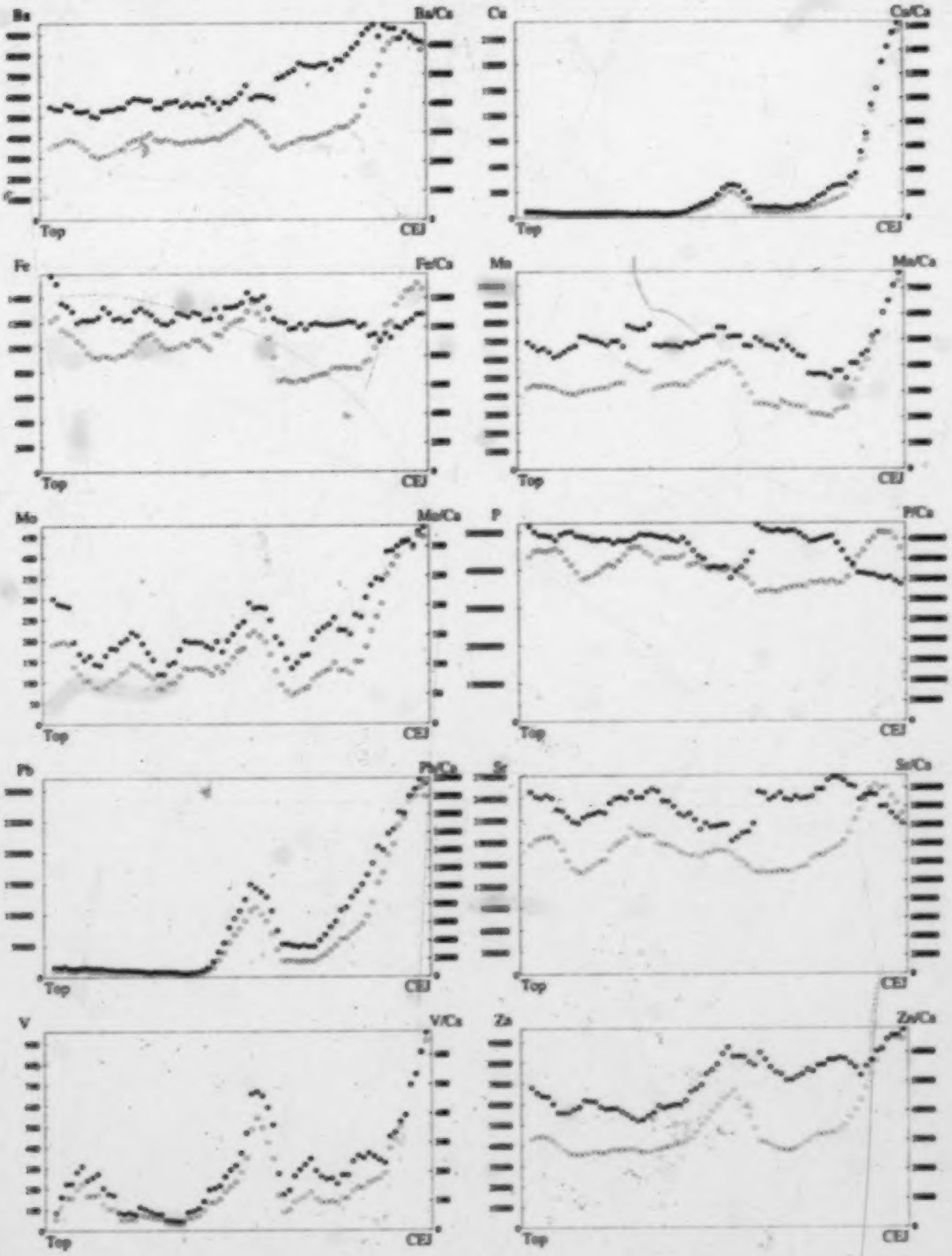

Fig. 5.22b: INDIVIDUAL A: Element and element/Ca ratios for combined lines on the right lateral incisor (A-Ri2). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

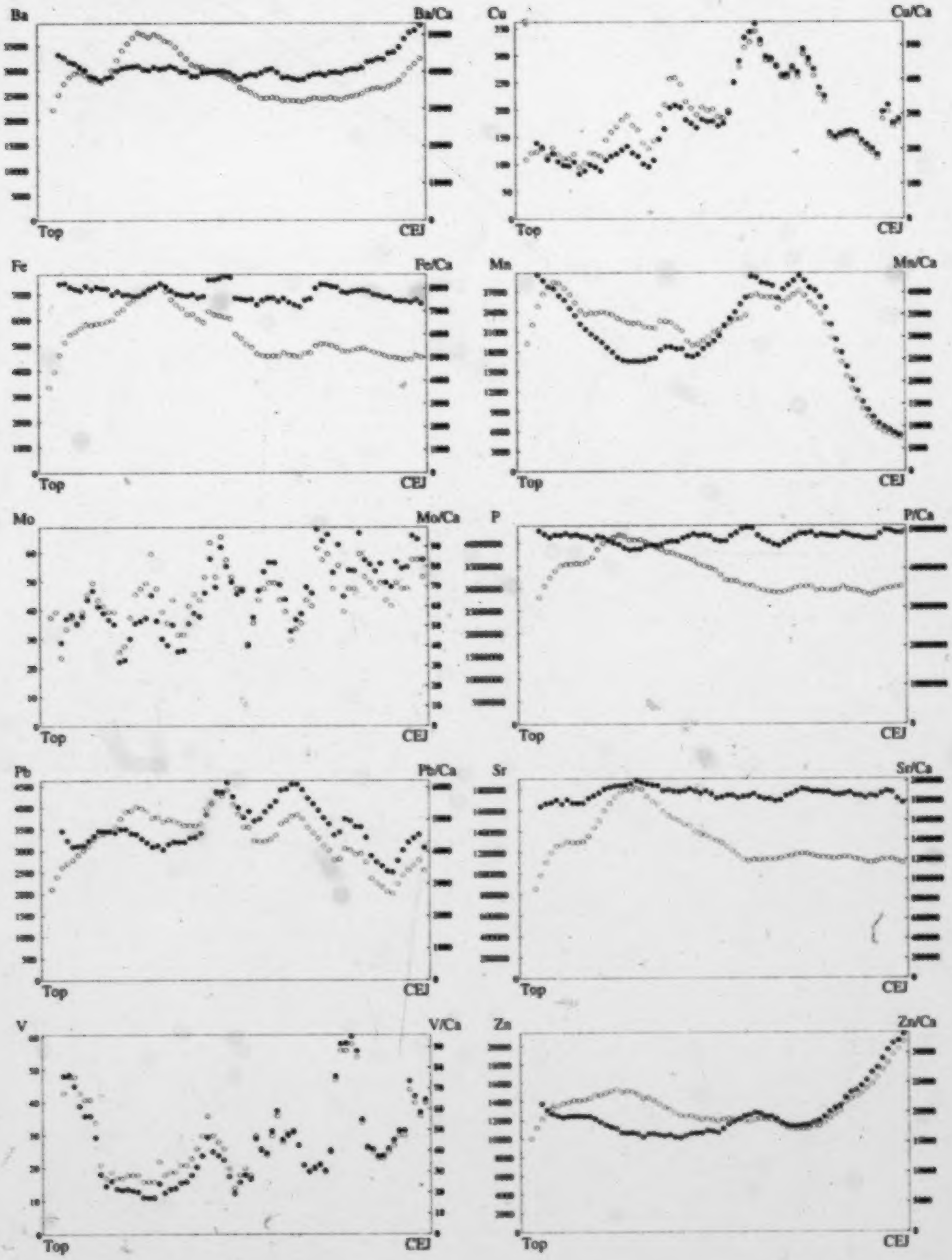

Fig. 5.22c: INDIVIDUAL A: Element and element/Ca ratios for line 1 on the right first molar (A-Rm1). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

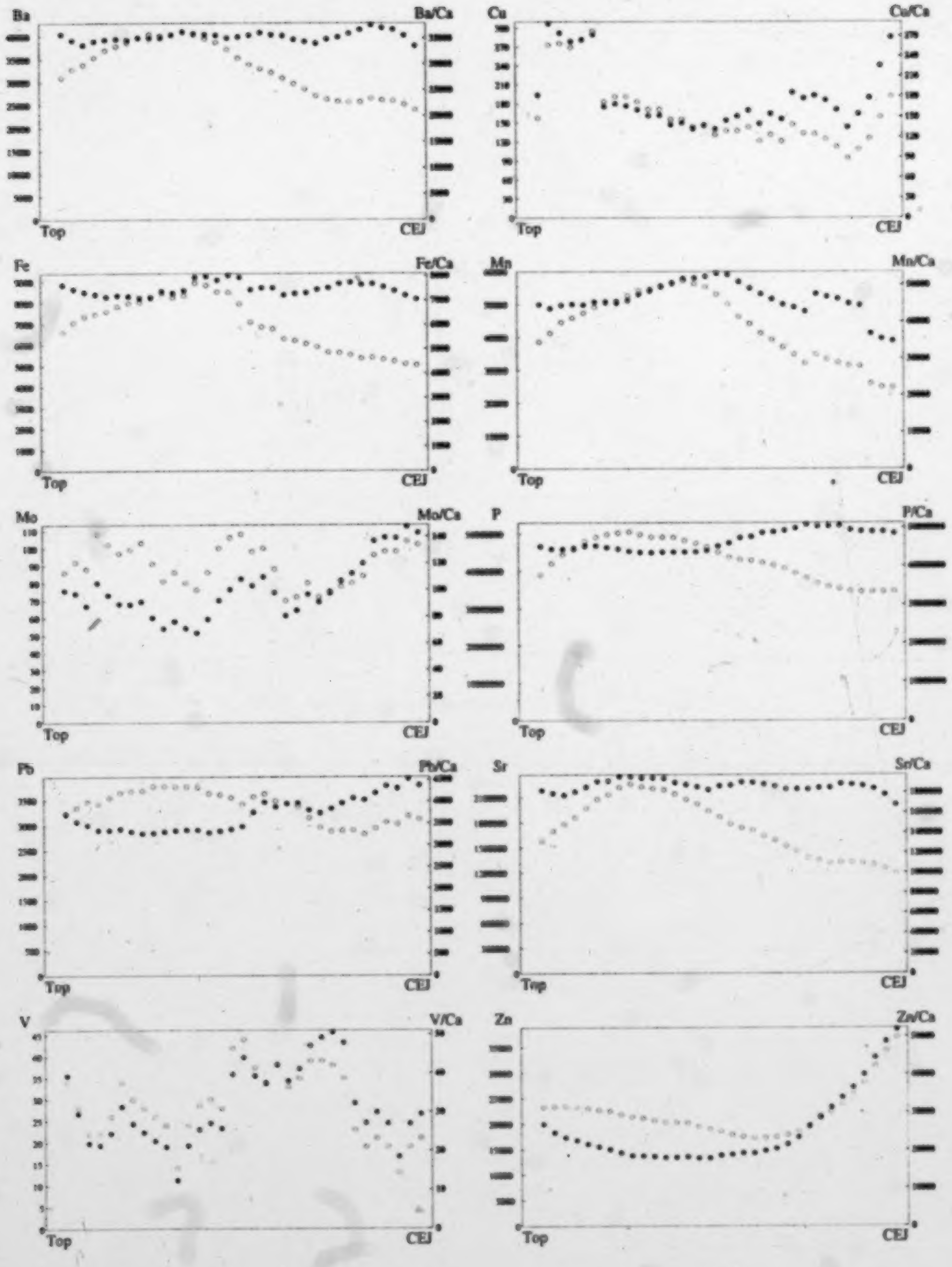

Fig. 5.22d: INDIVIDUAL A: Element and element/Ca ratios for line 2 on the right first molar (A-Rm1). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

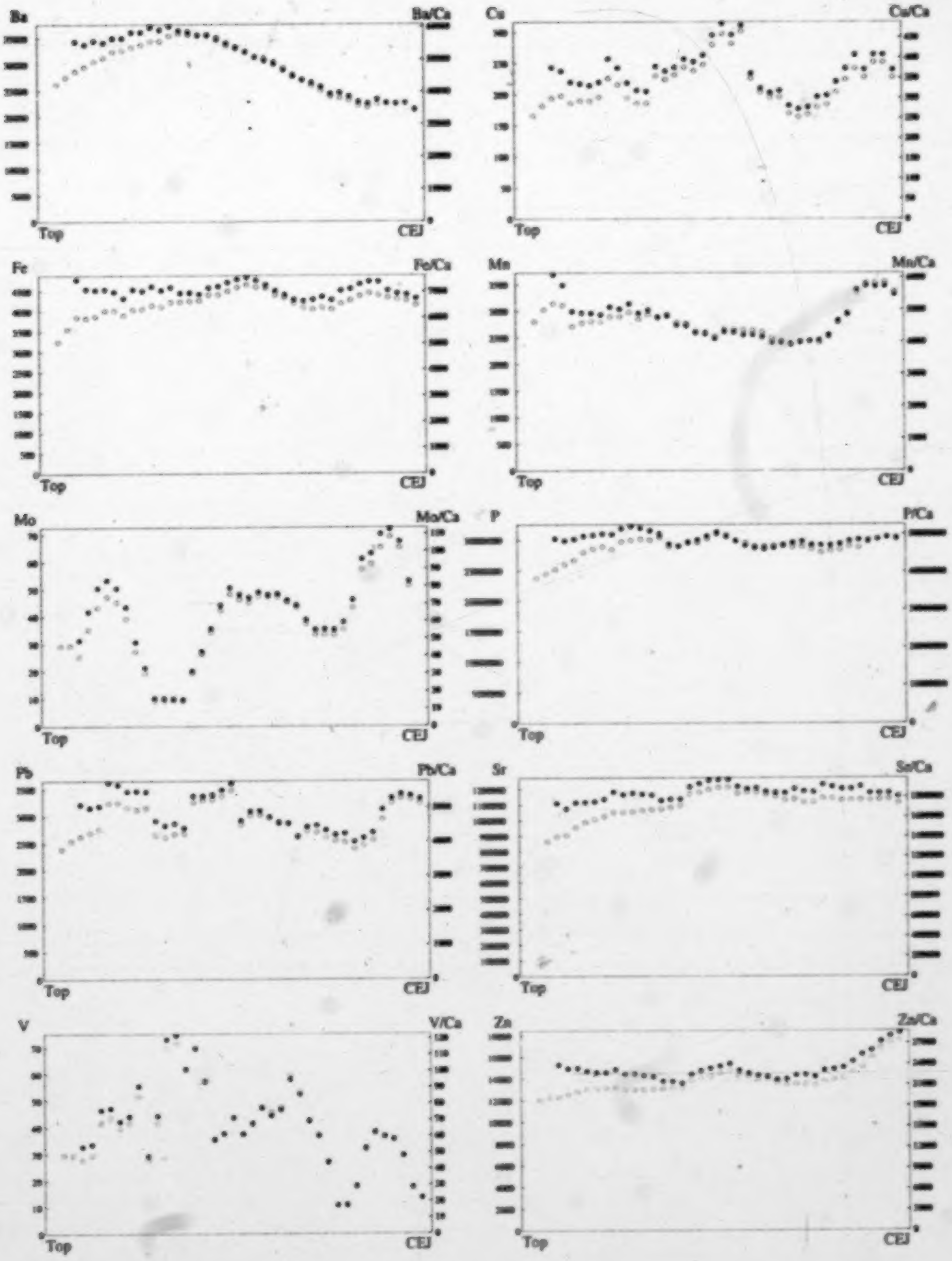

Fig. 5.22e: INDIVIDUAL A: Element and element/Ca ratios for line 1 on the right second molar (A-Rm2). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

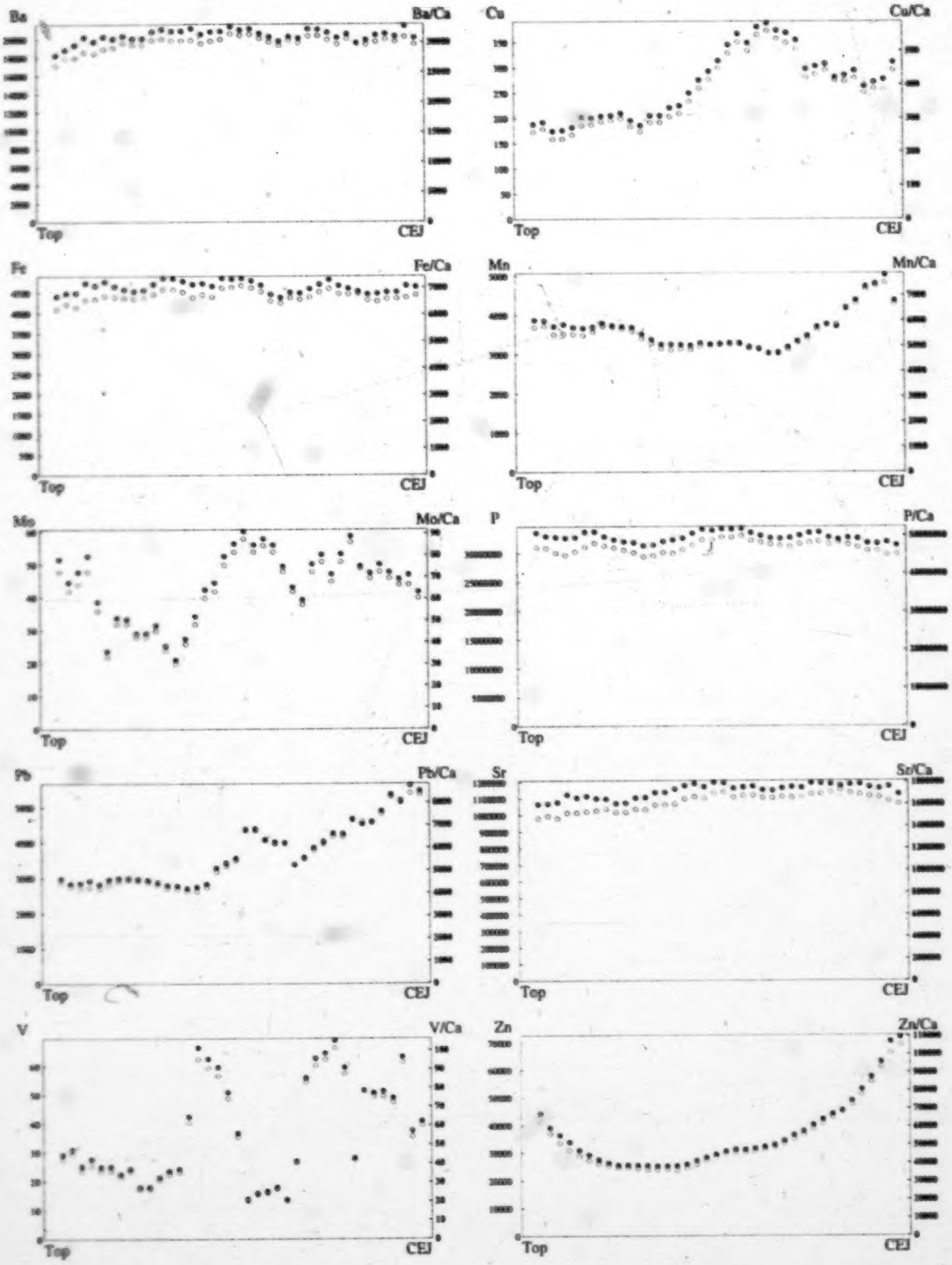

Fig. 5.22f: INDIVIDUAL A: Element and element/Ca ratios for line 2 on the right second molar (A-Rm2). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

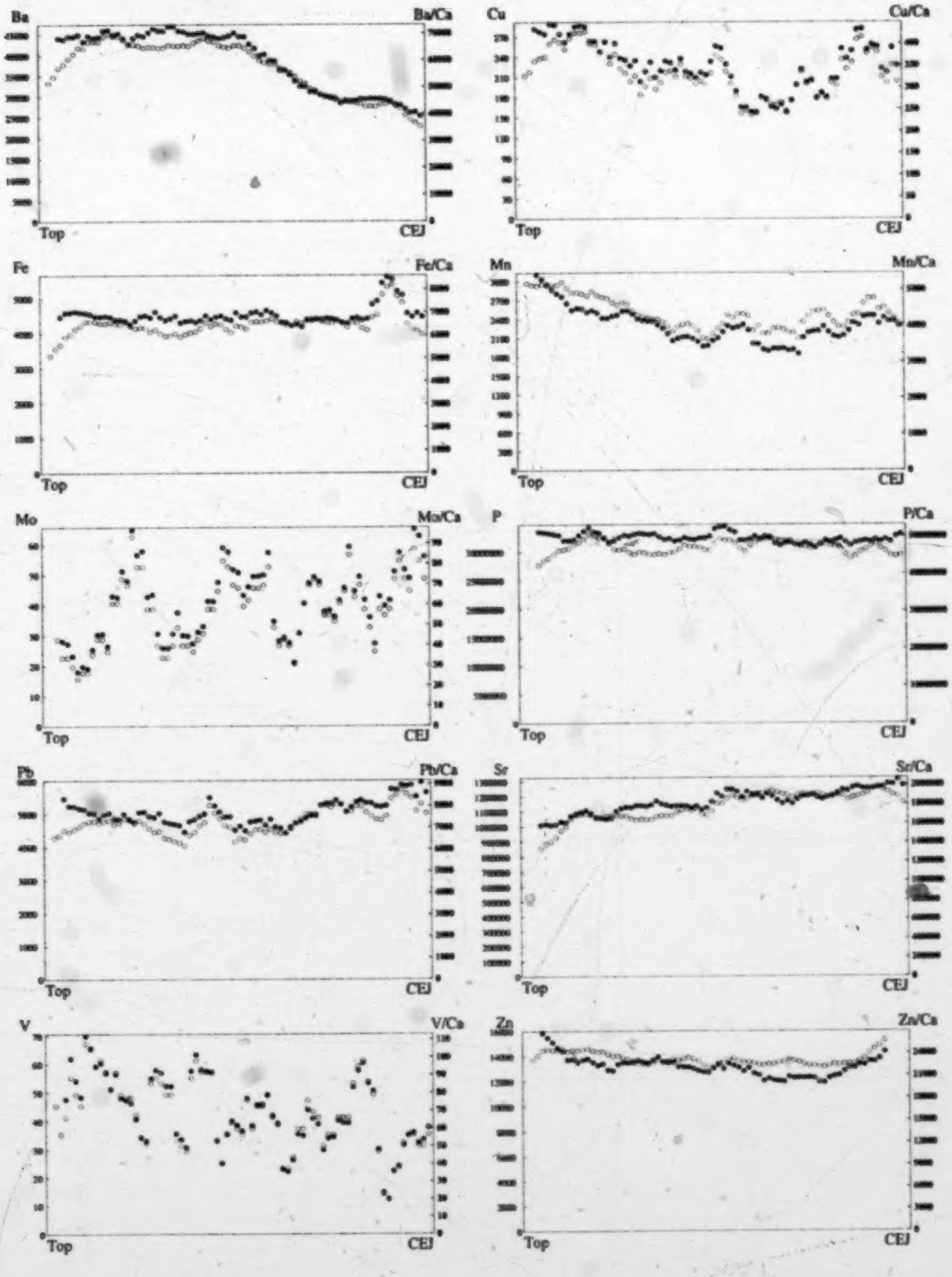

Fig. 5.22g: INDIVIDUAL A: Element and element/Ca ratios for line 4 on the right second molar (A-Rm2). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

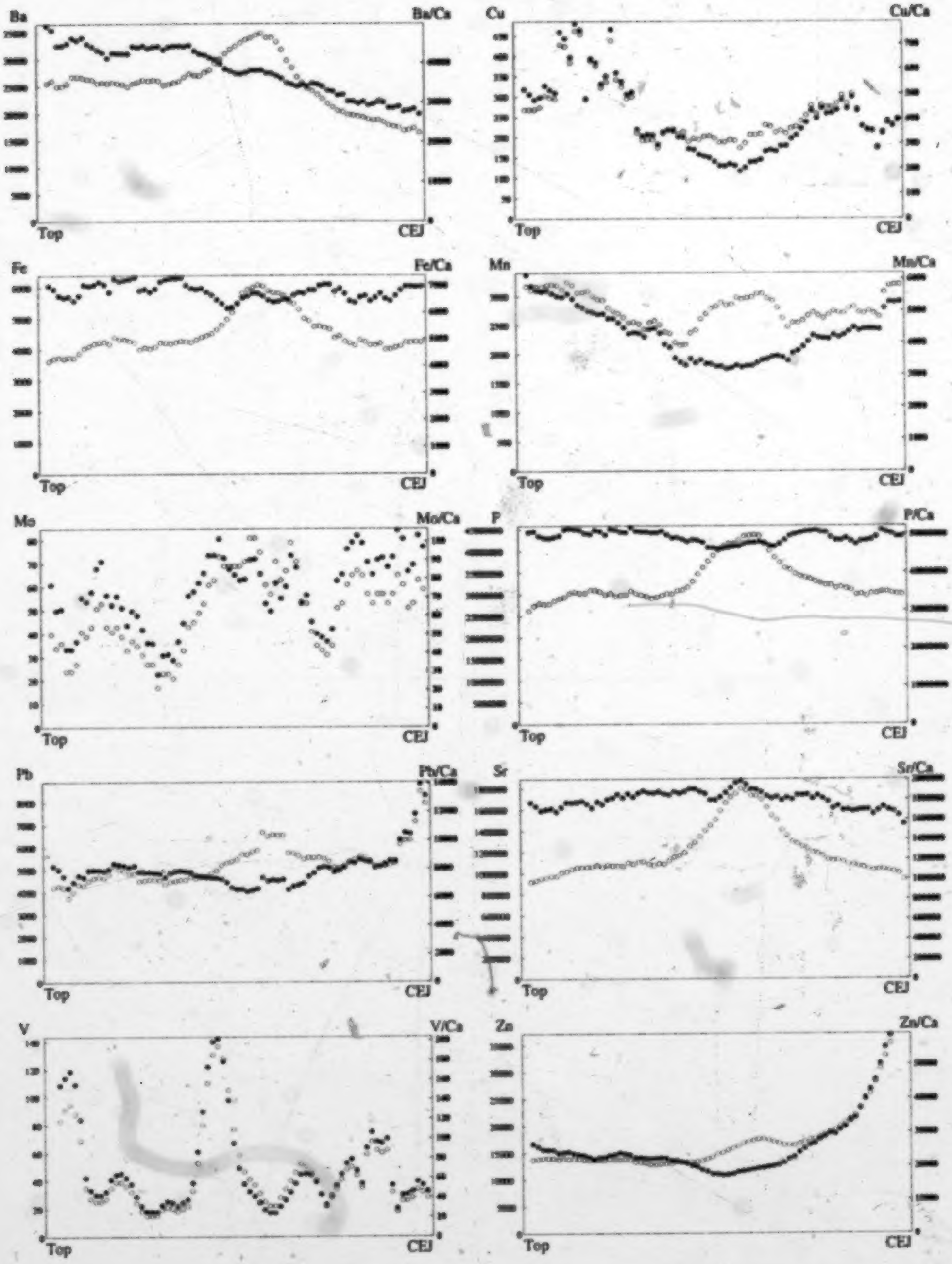

Fig. 5.22h: INDIVIDUAL A: Element and element/Ca ratios for line 5 on the right second molar (A-Rm2). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

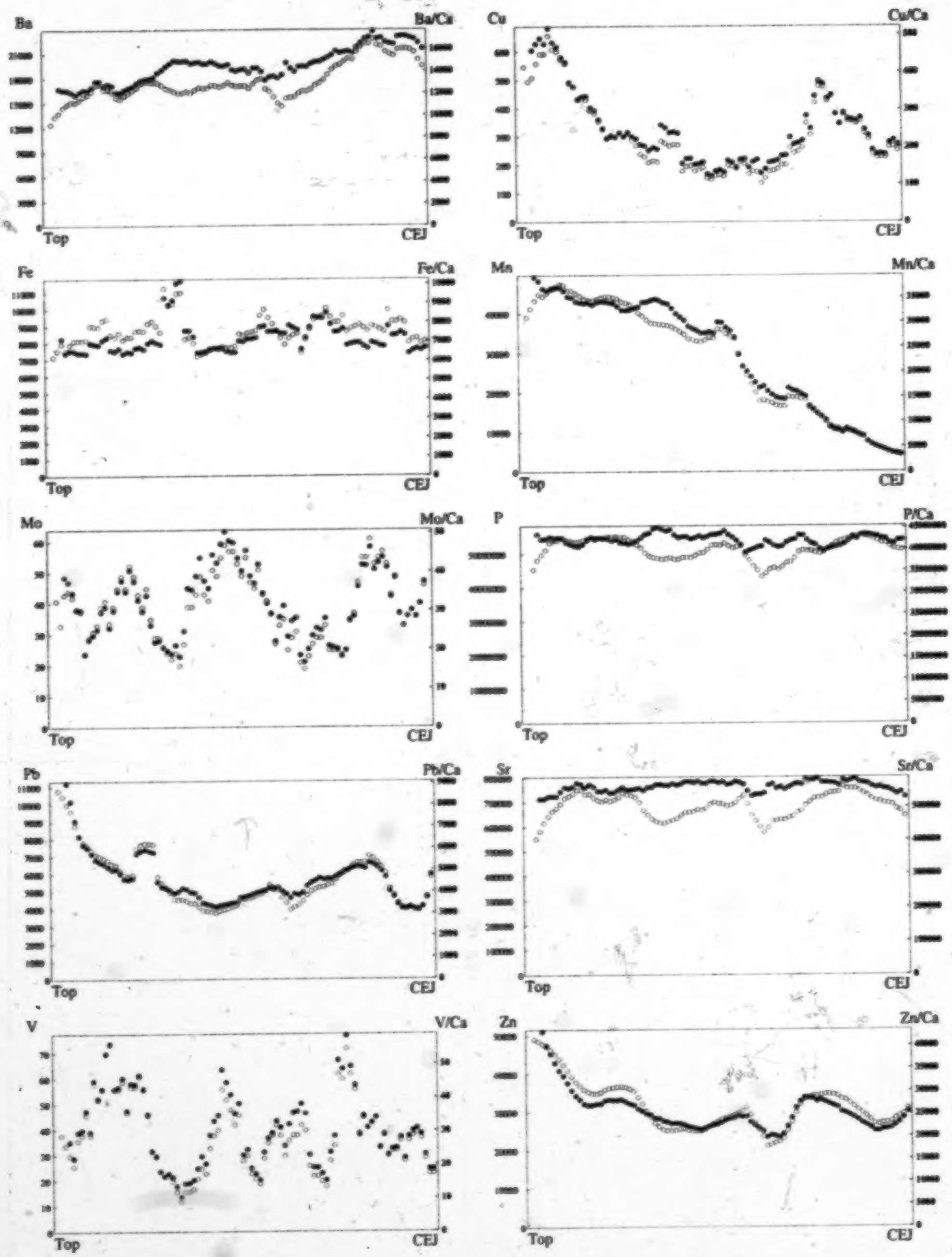

Fig. 5.23a: INDIVIDUAL B: Element and element/Ca ratios for combined lines on the right central incisor (B-Ri1). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

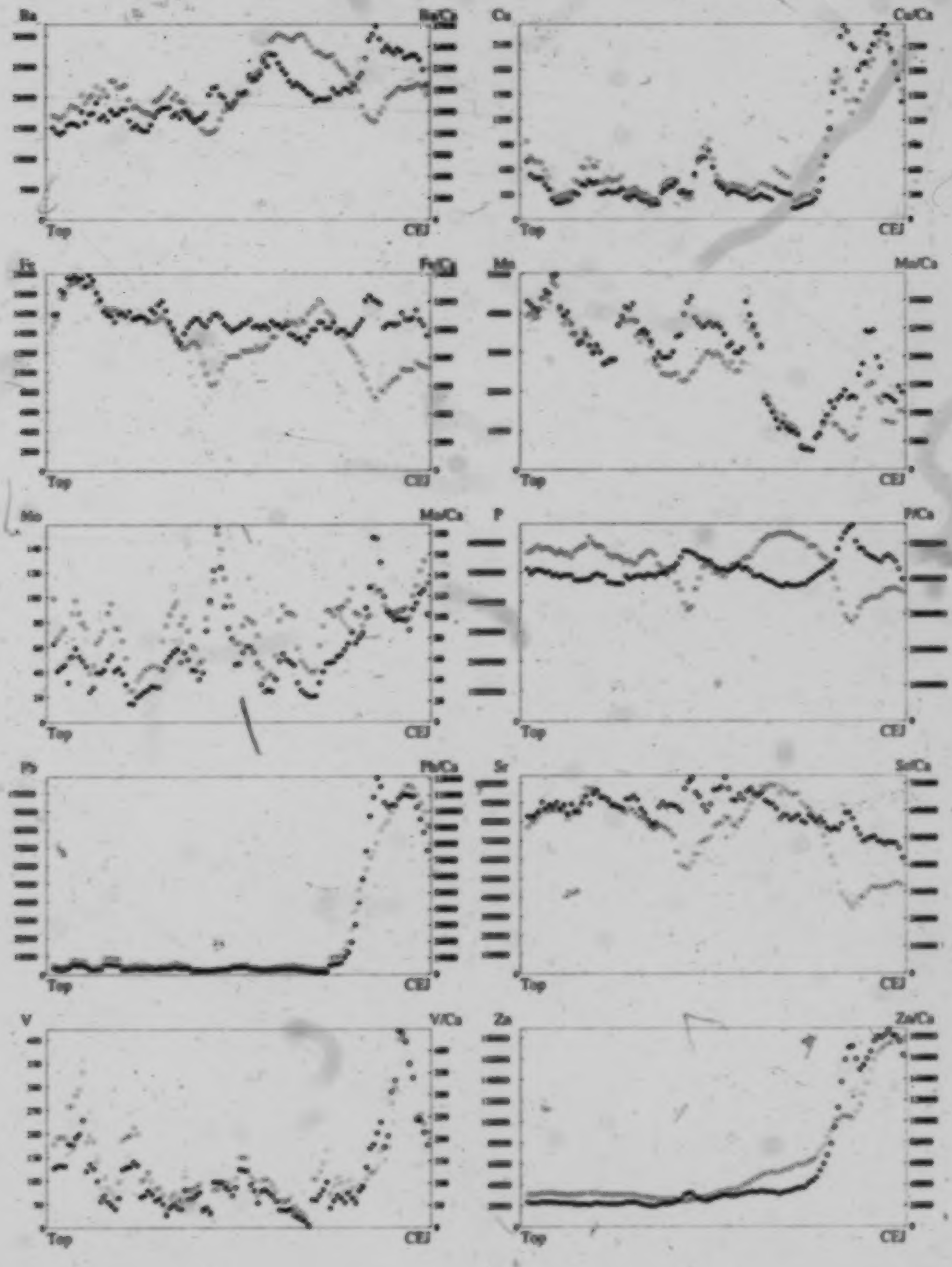

Fig. 5.23b: INDIVIDUAL B: Element and element/Ca ratios for combined lines on the right lateral incisor (B-Ri2). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

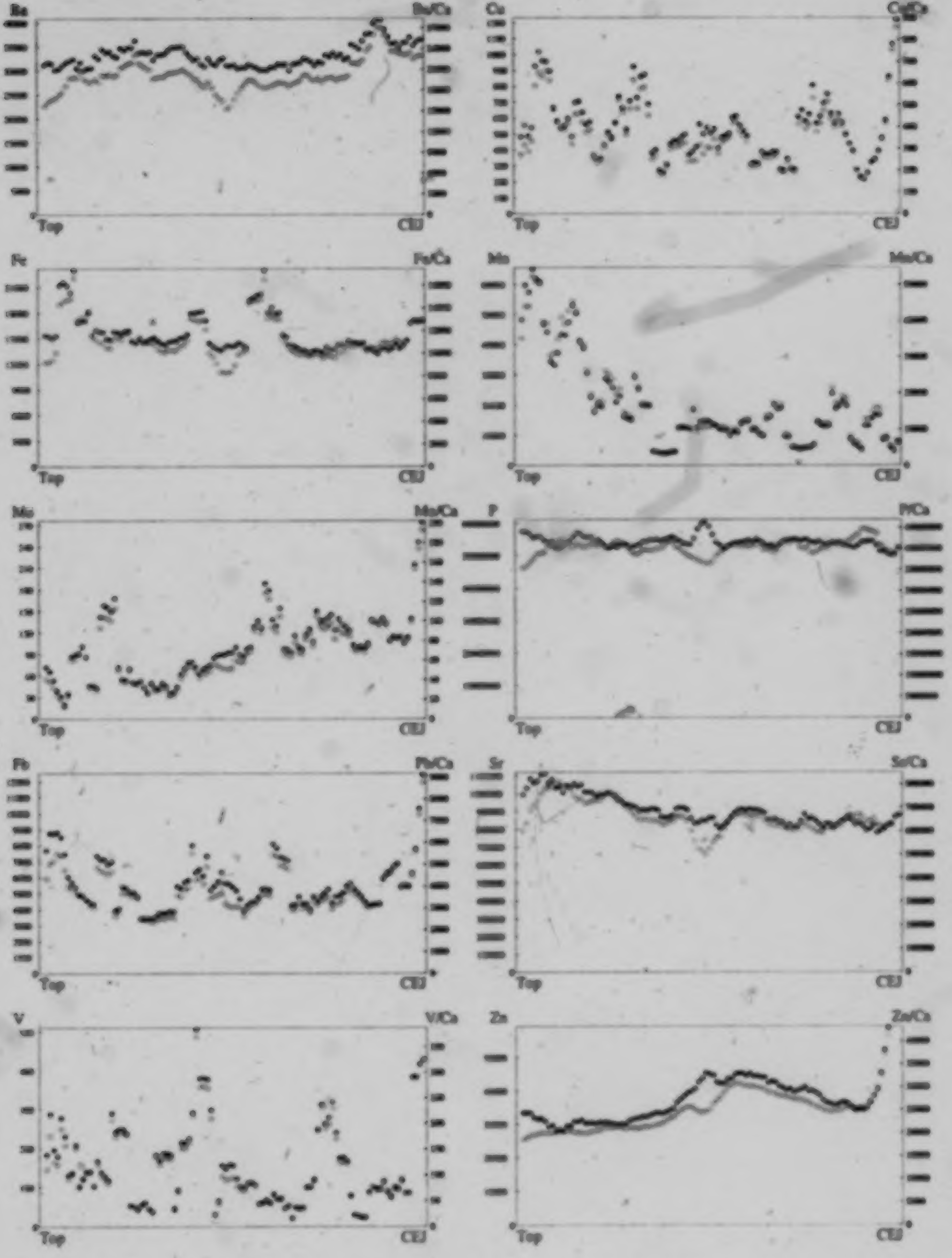

Fig. 5.23c: INDIVIDUAL B: Element and element/Ca ratios for combined lines on the right canine (B-Rc). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

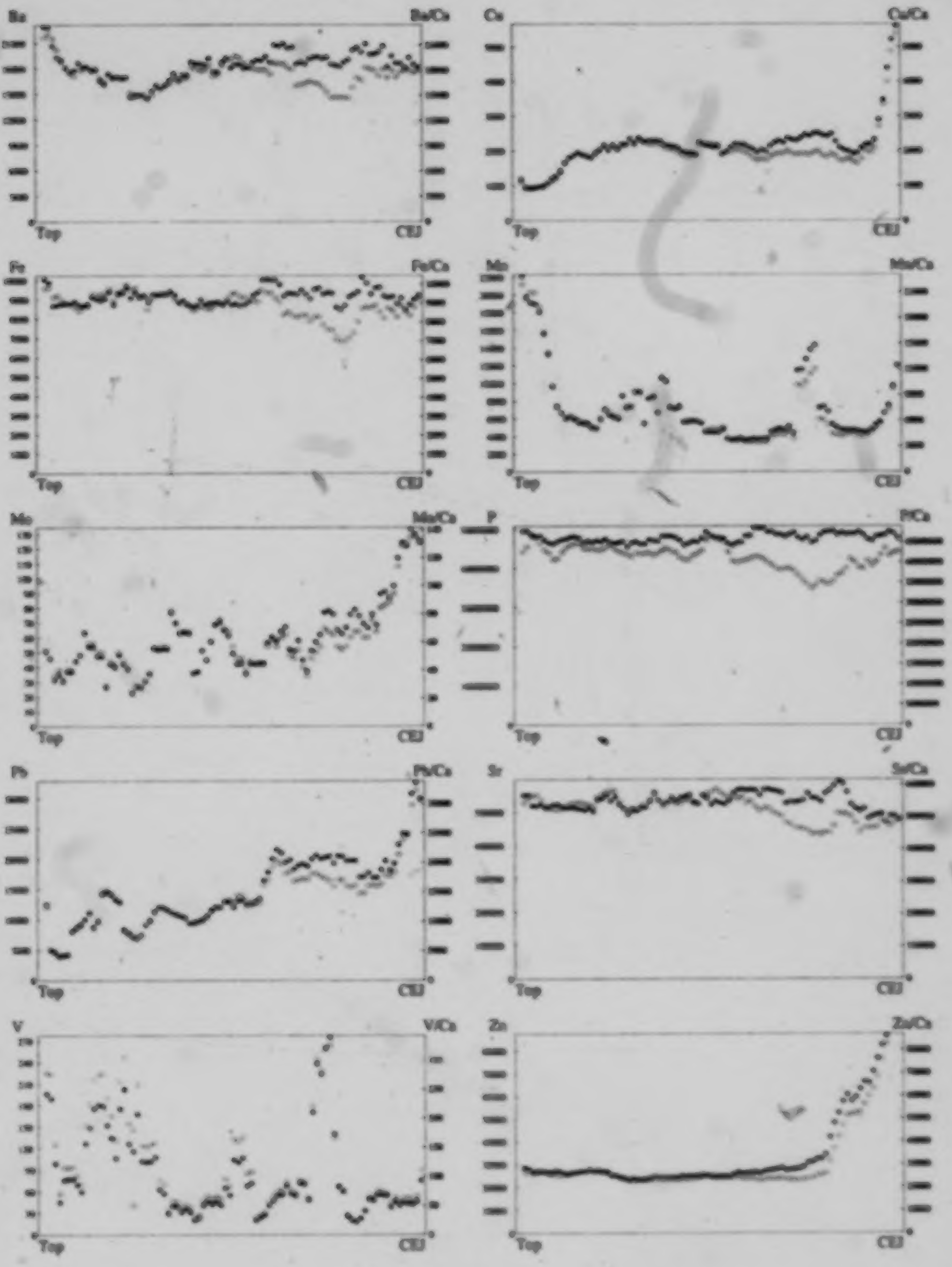

Fig. 5.23d: INDIVIDUAL B: Element and element/Ca ratios for combined lines on the right first molar (B-Rm1). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

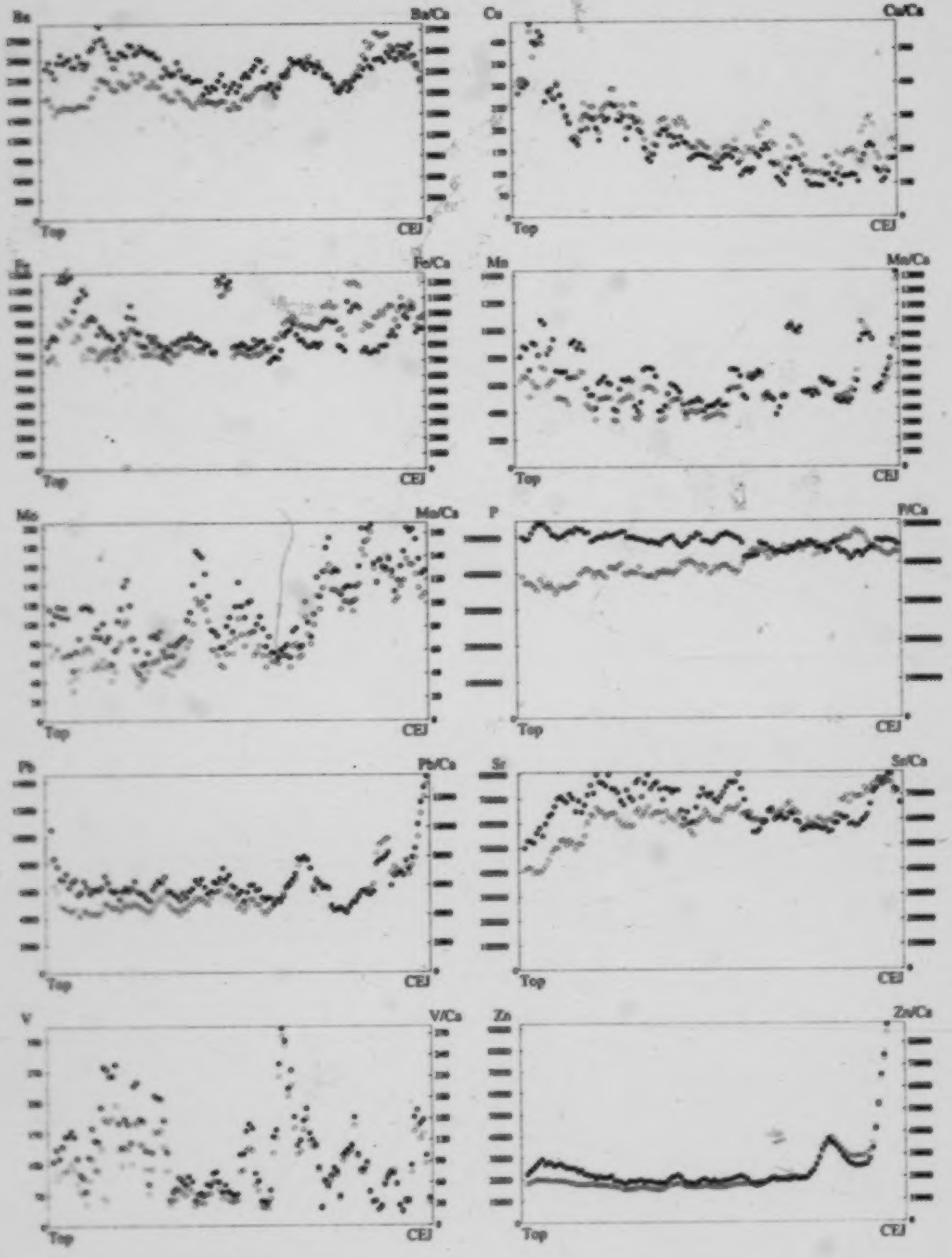

Fig. 5.23e: INDIVIDUAL B: Element and element/Ca ratios for combined lines on the right second molar (B-Rm2). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

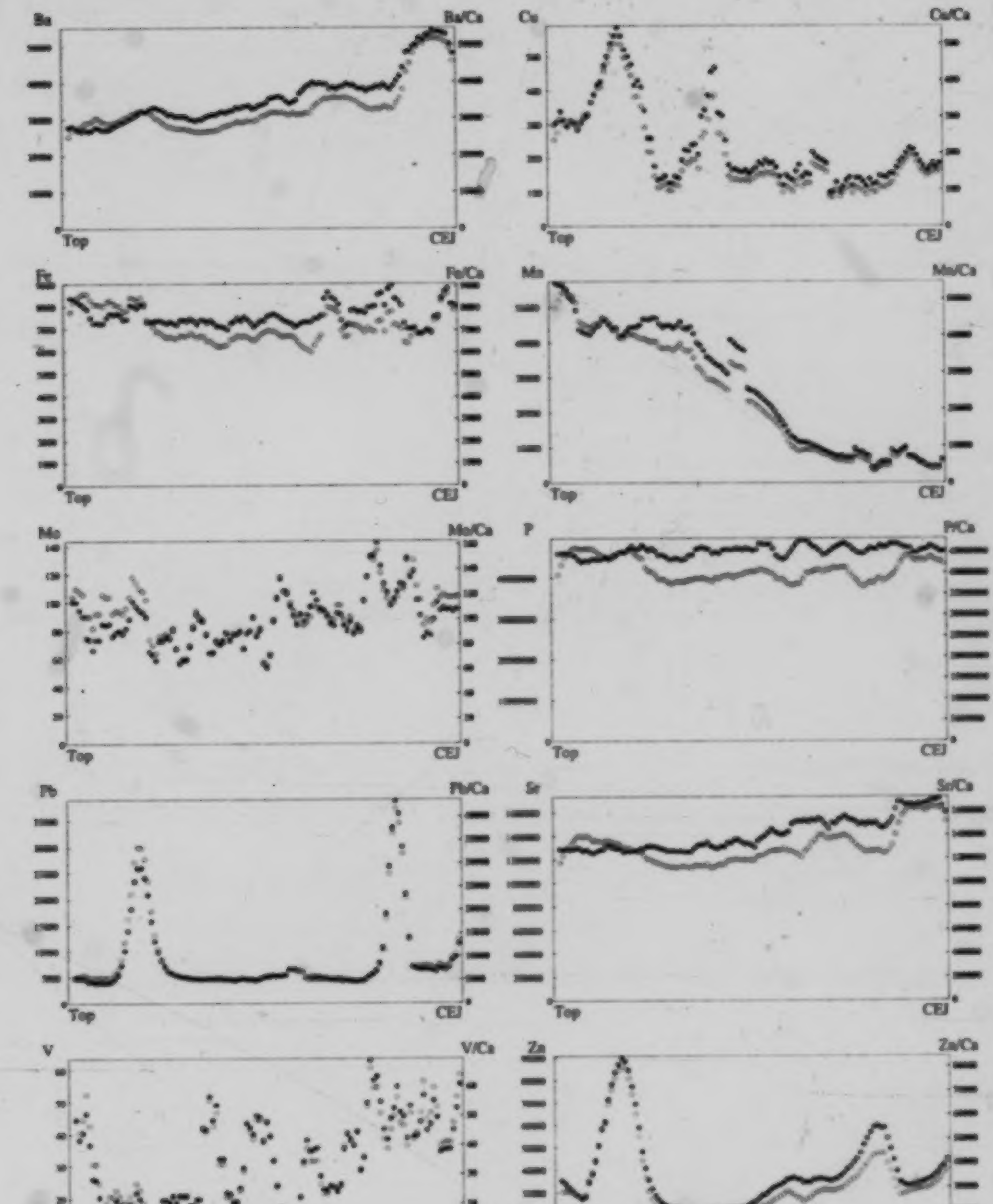

Fig. 5.24a: INDIVIDUAL C: Element and element/Ca ratios for combined lines 1, 3 and 5 on the right central incisor (C-Ri1). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

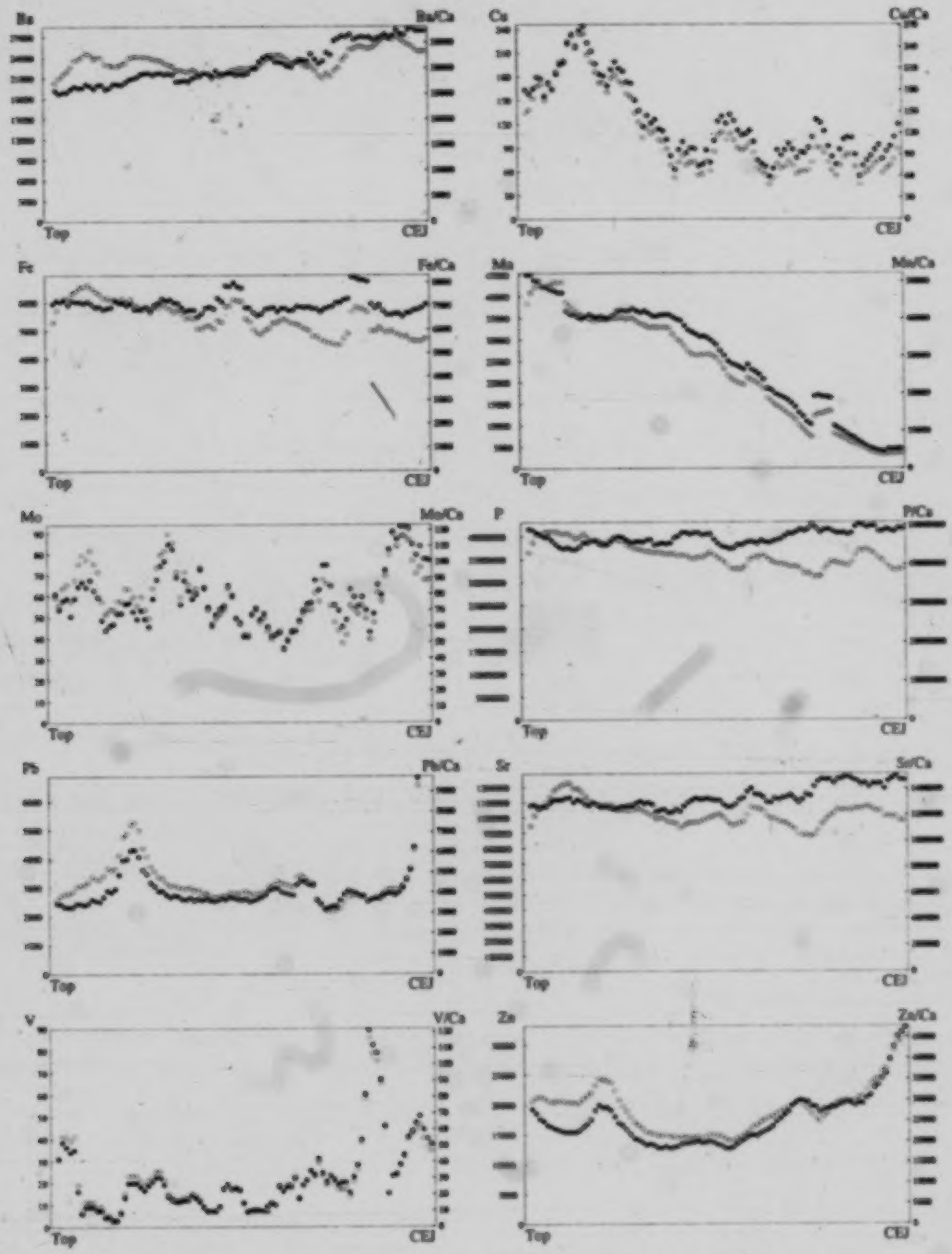

Fig. 5.24b: INDIVIDUAL C: Element and element/Ca ratios for combined lines 2 and 4 on the right central incisor (C-Ri1). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

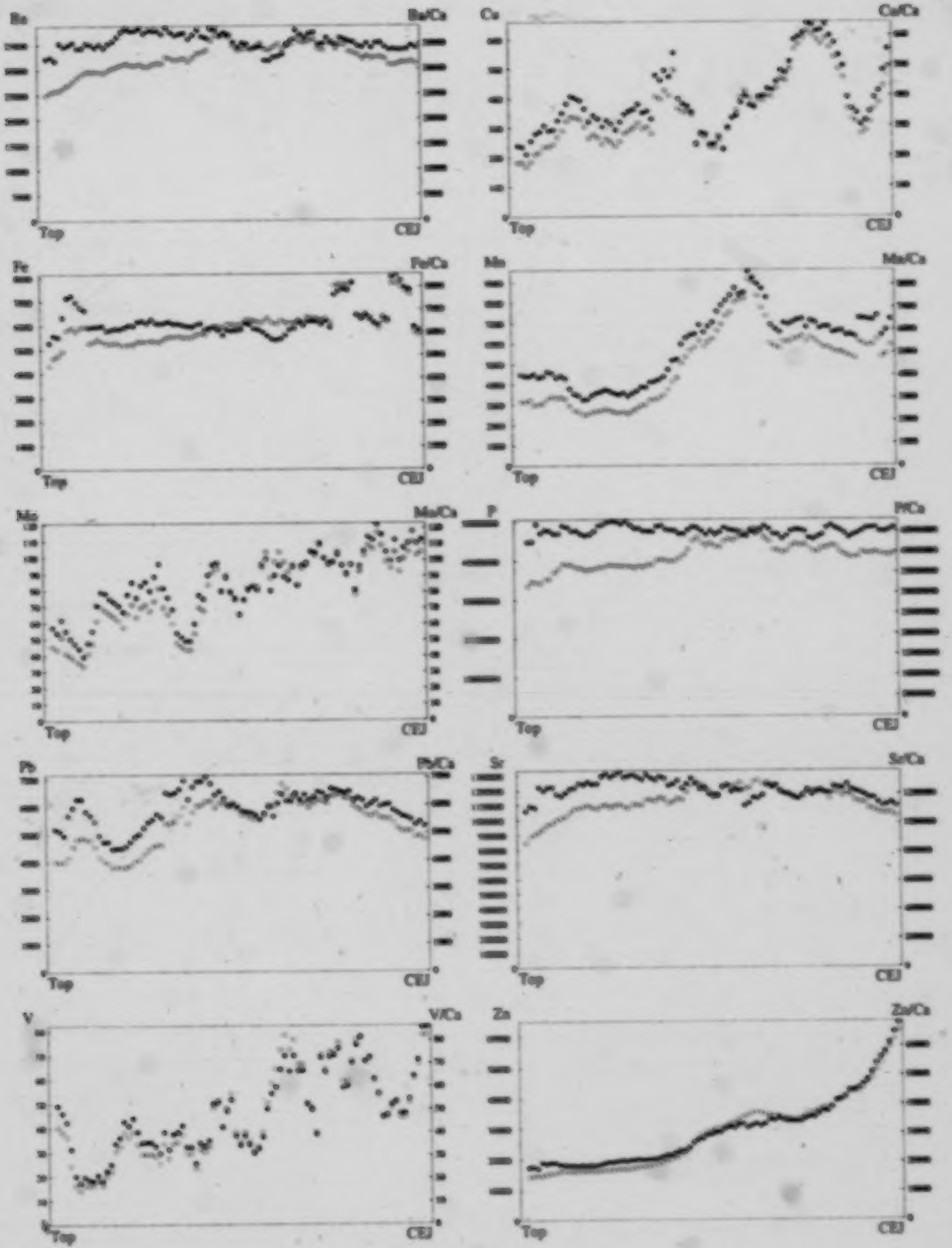

Fig. 5.24c: INDIVIDUAL C: Element and element/Ca ratios for line 1 on the left canine (C-Lc). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

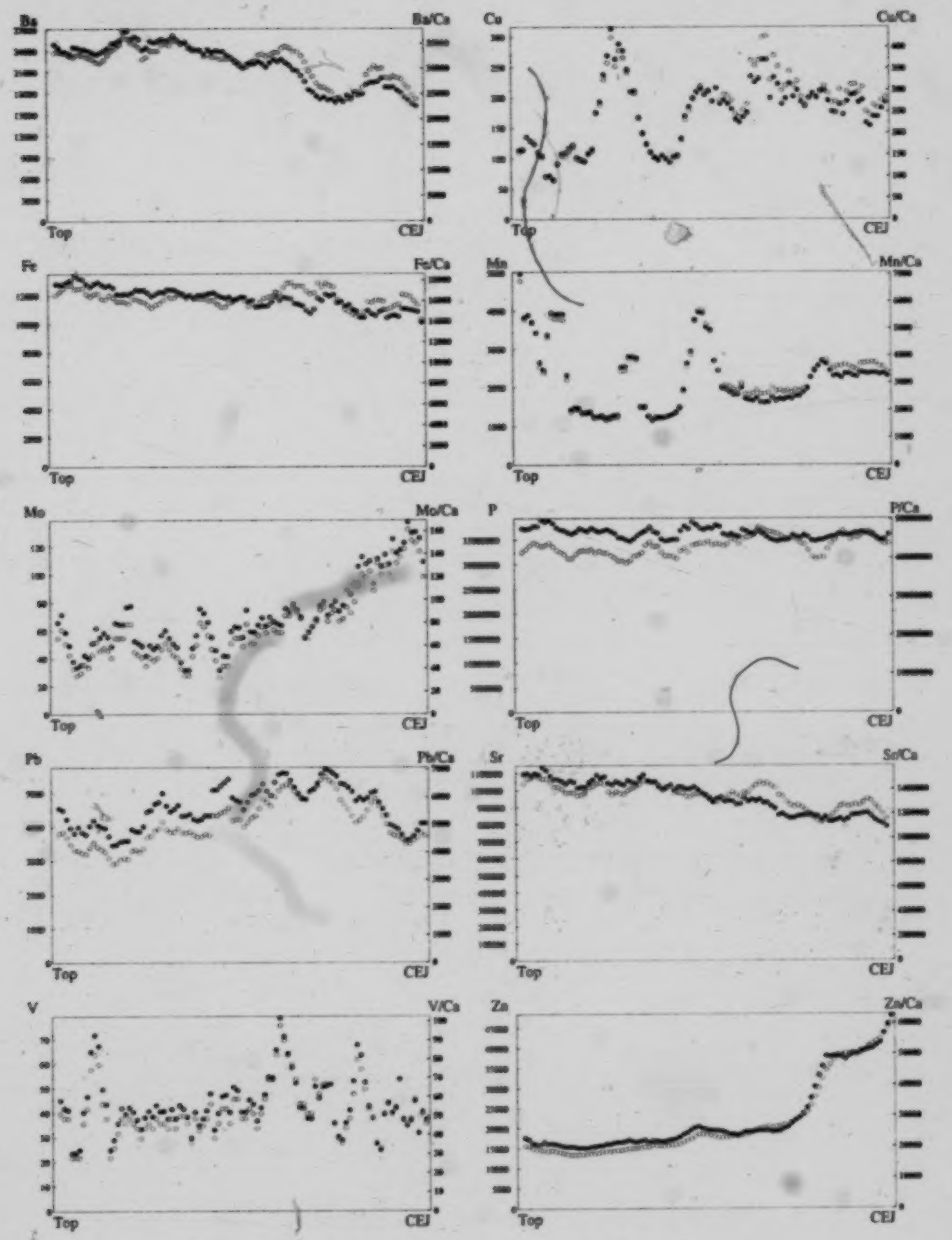

Fig. 5.24d: INDIVIDUAL C: Element and element/Ca ratios for combined lines on the left first molar (C-Lm1). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

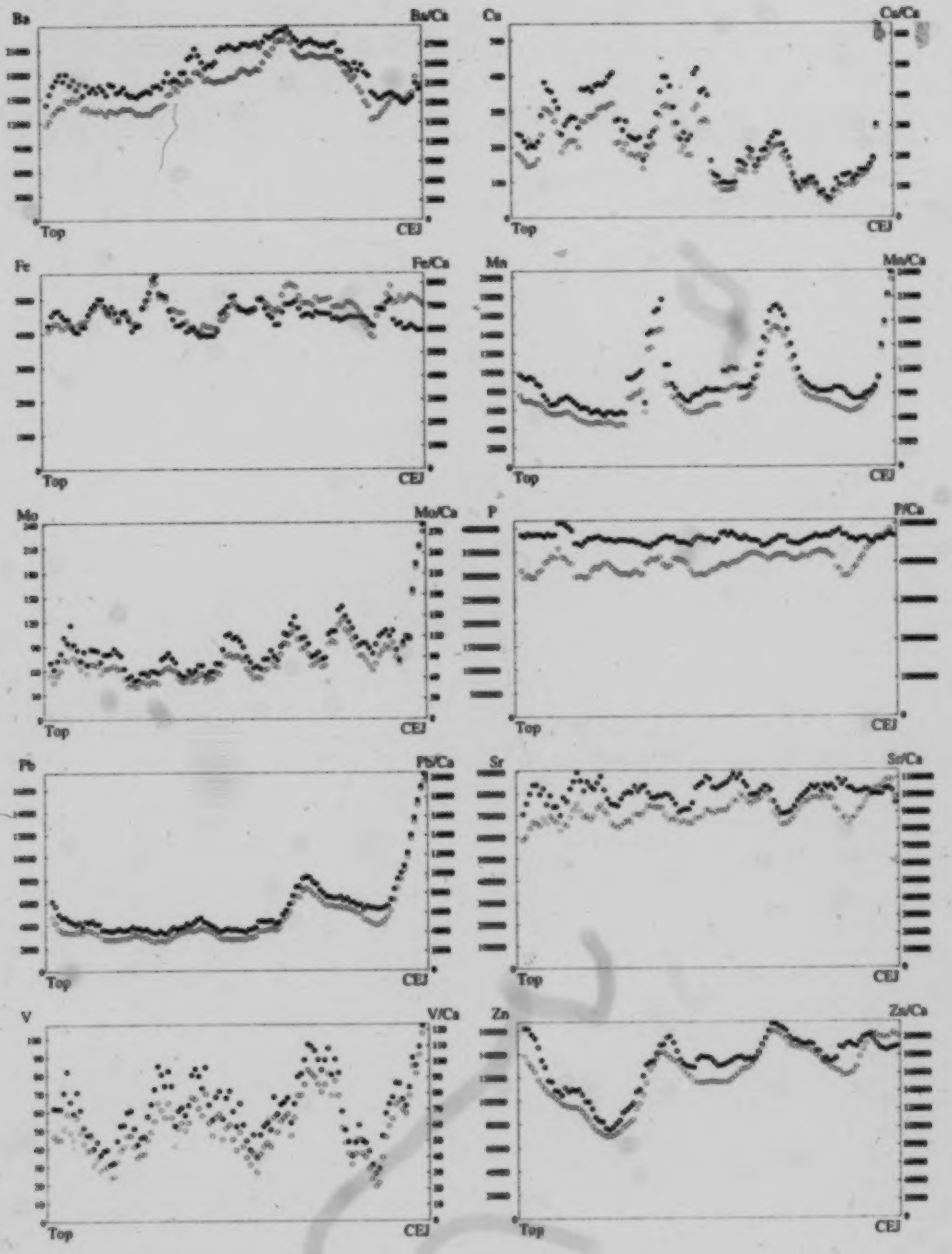

Fig. 5.24e: INDIVIDUAL C: Element and element/Ca ratios for combined lines on the left second molar (C-Lm2). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

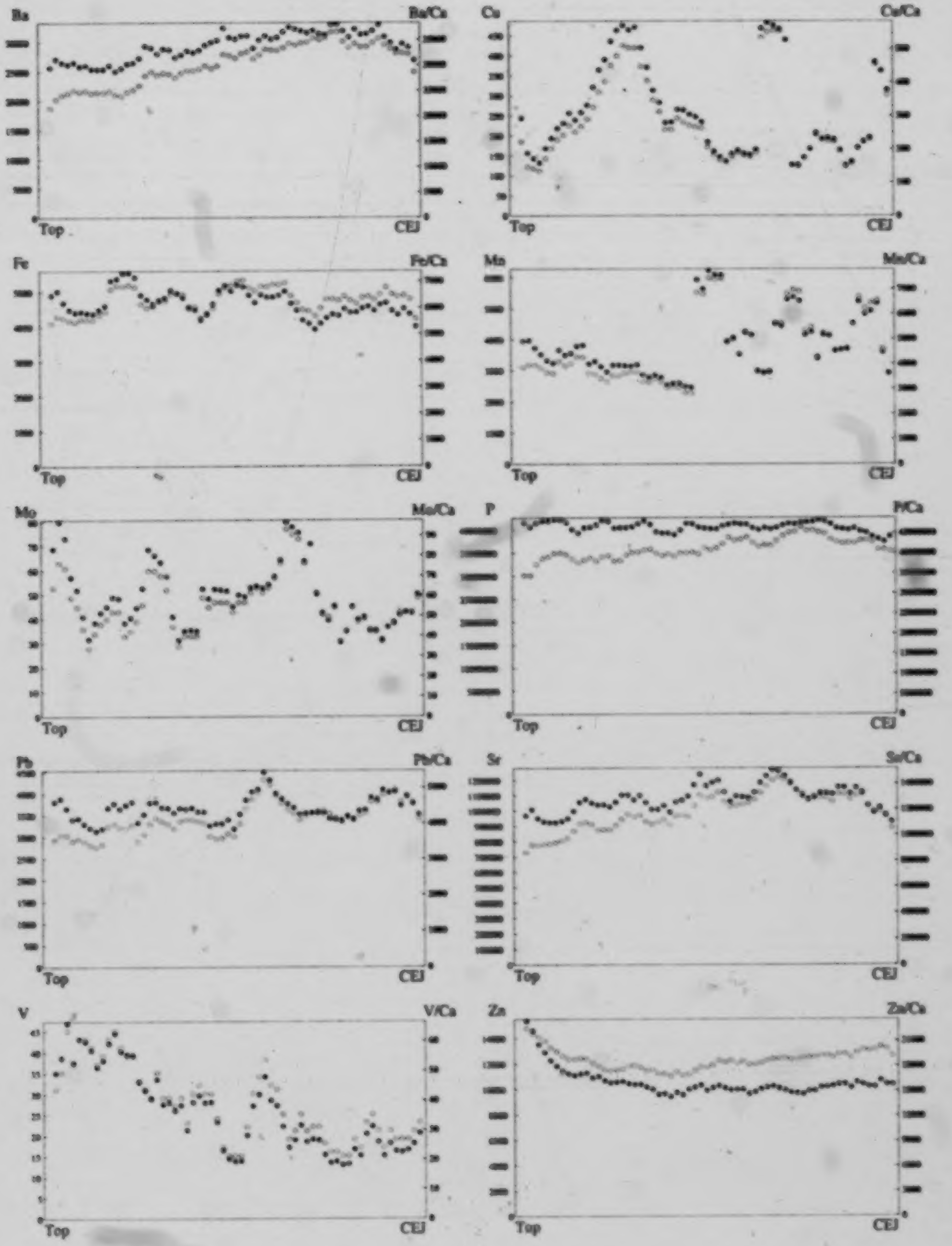

Fig. 5.24f: INDIVIDUAL C: Element and element/Ca ratios for line 5 on the left second molar (C-Lm2). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

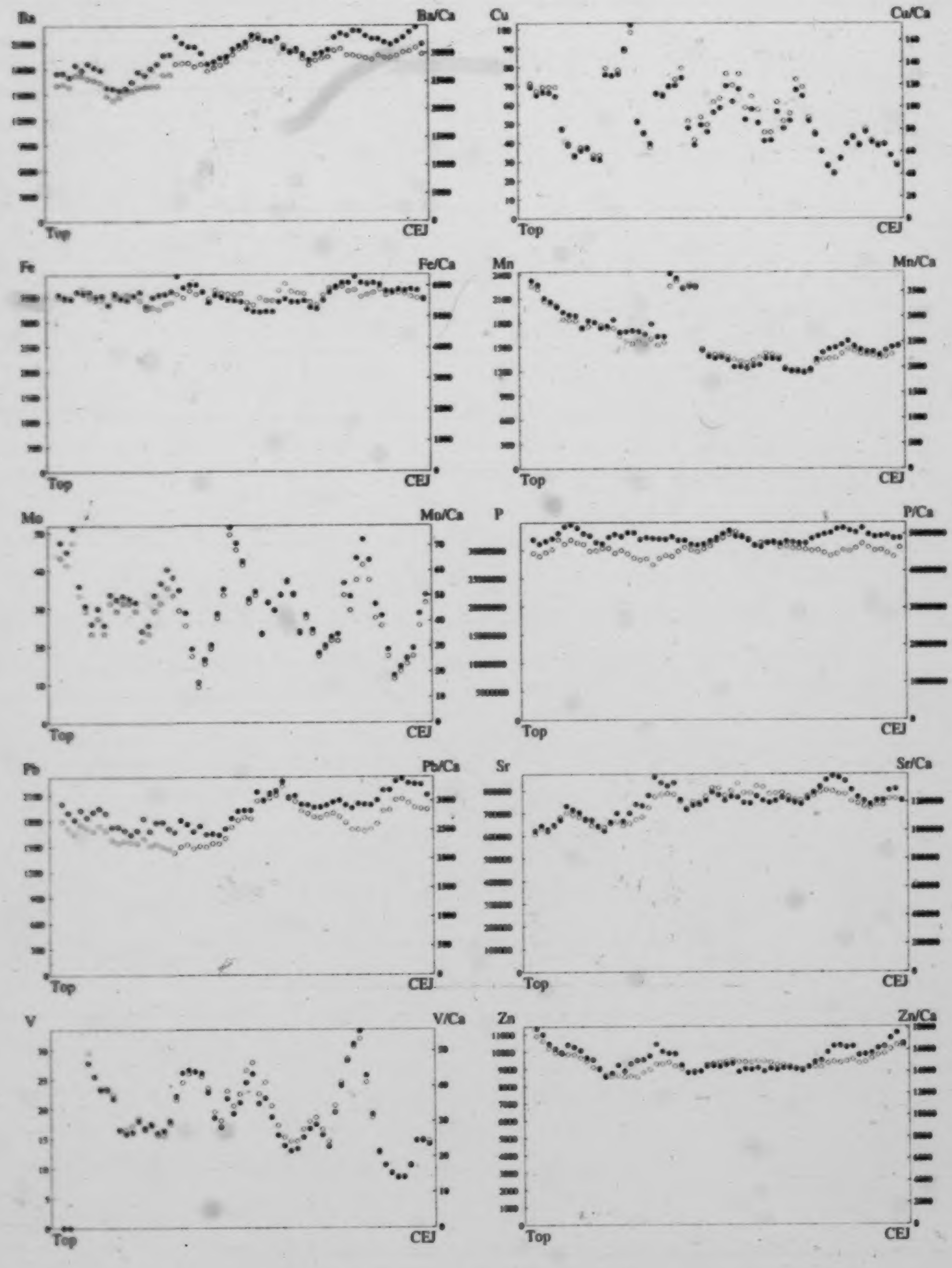

Fig. 5.24g: INDIVIDUAL C: Element and element/Ca ratios for line 6 on the left second molar (C-Lm2). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

Differences between teeth of same individual

The developmental periods for the deciduous tooth types show a substantial overlap. Therefore, one would expect similarities in enamel composition for the different teeth within a dentition. To compare the composition of the different teeth, box plots were created (Figs. 5.25 – 5.27). These graphs show that the concentrations of all elements are indeed mostly comparable. The only notable exceptions are the concentration of Mn in A-Rm2 (Fig. 5.25 a), and the concentration of Fe in the C-Lm1 (Fig. 5.27 a). Given the fact that all deciduous teeth show substantial overlap in their developmental time, these observations of consistently higher concentrations of Fe and Mn in one tooth are difficult to explain in terms of dietary intake (see below).

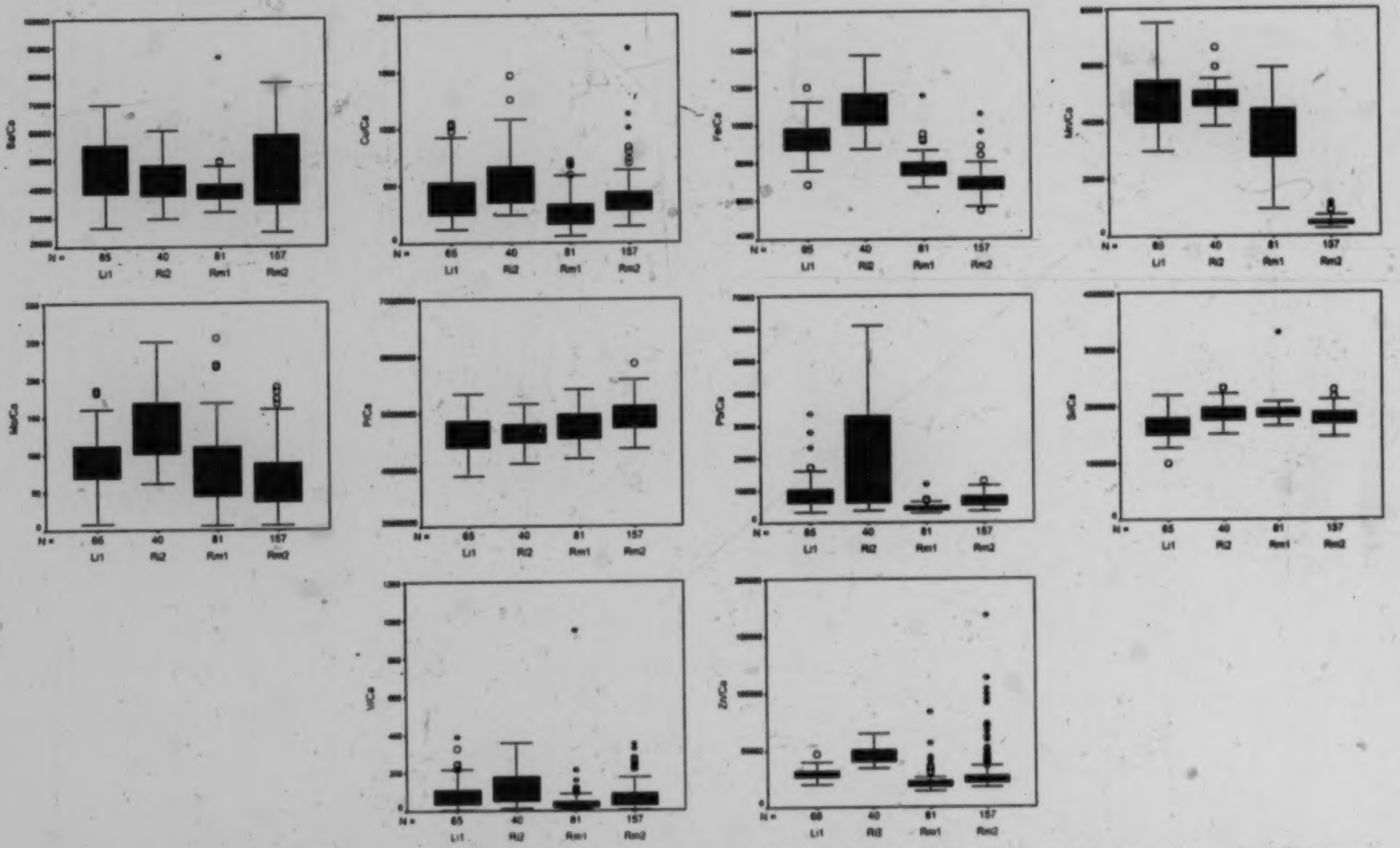

Fig. 5.25a: Boxplots for individual A, showing the distributions of the Ca ratios for each tooth separately. Along the x-axis are shown the number of points measured on each line (N) and the teeth. Li1 = left first incisor; Ri2 = right second incisor; Rm1 = right first molar; Rm2 = right second molar. ○= outlier, *=extreme value.

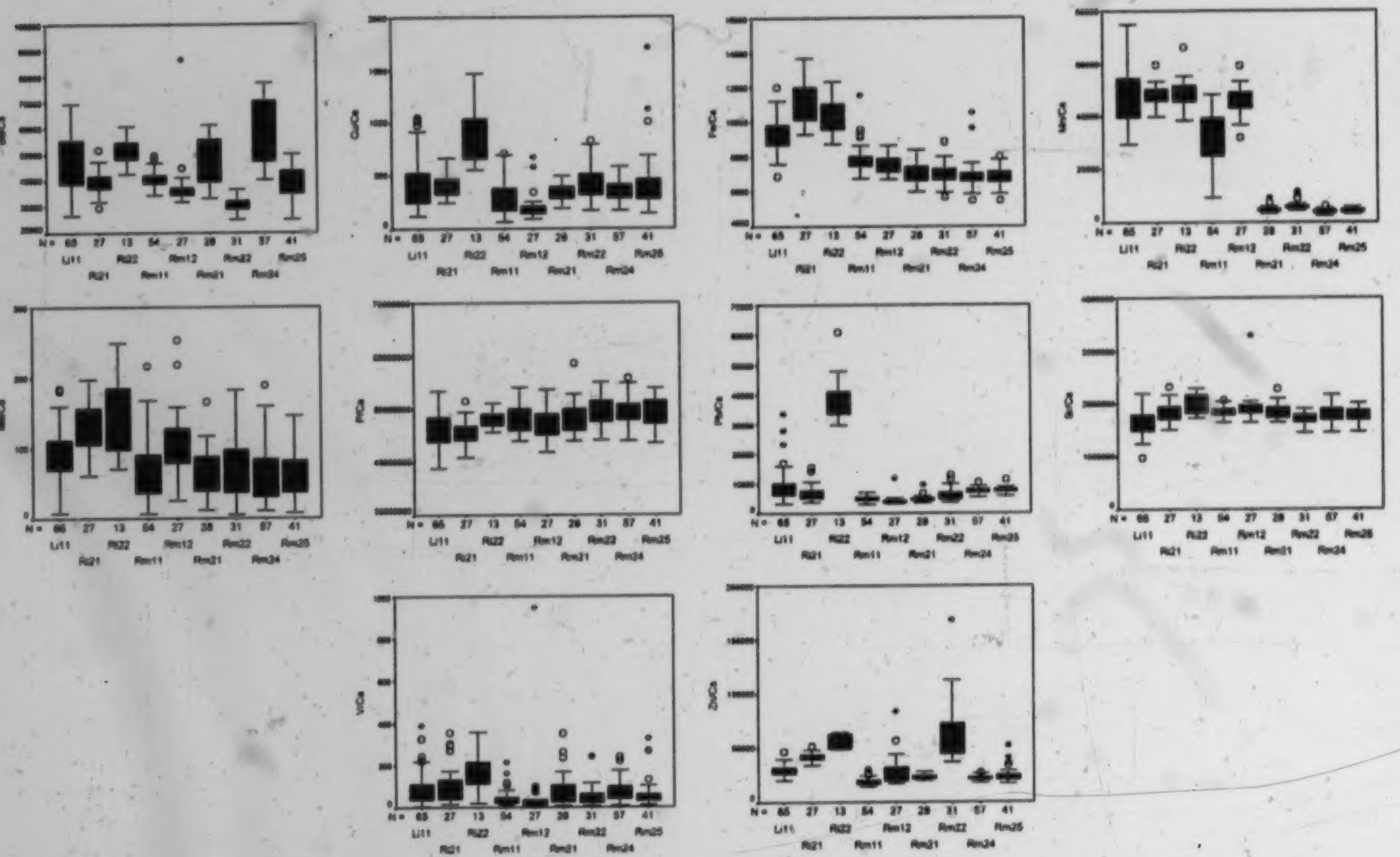

*Fig. 5.25b: Boxplots for individual A, showing the distributions of the Ca ratios for individual lines separately. Along the x-axis are shown the number of points measured on each line (N), the teeth and the longitudinal lines on each tooth. Li1 = left first incisor; Ri2 = right second incisor; Rm1 = right first molar; Rm2 = right second molar; Li11 = left first incisor, line 1, etc. ○= outlier, *=extreme value.*

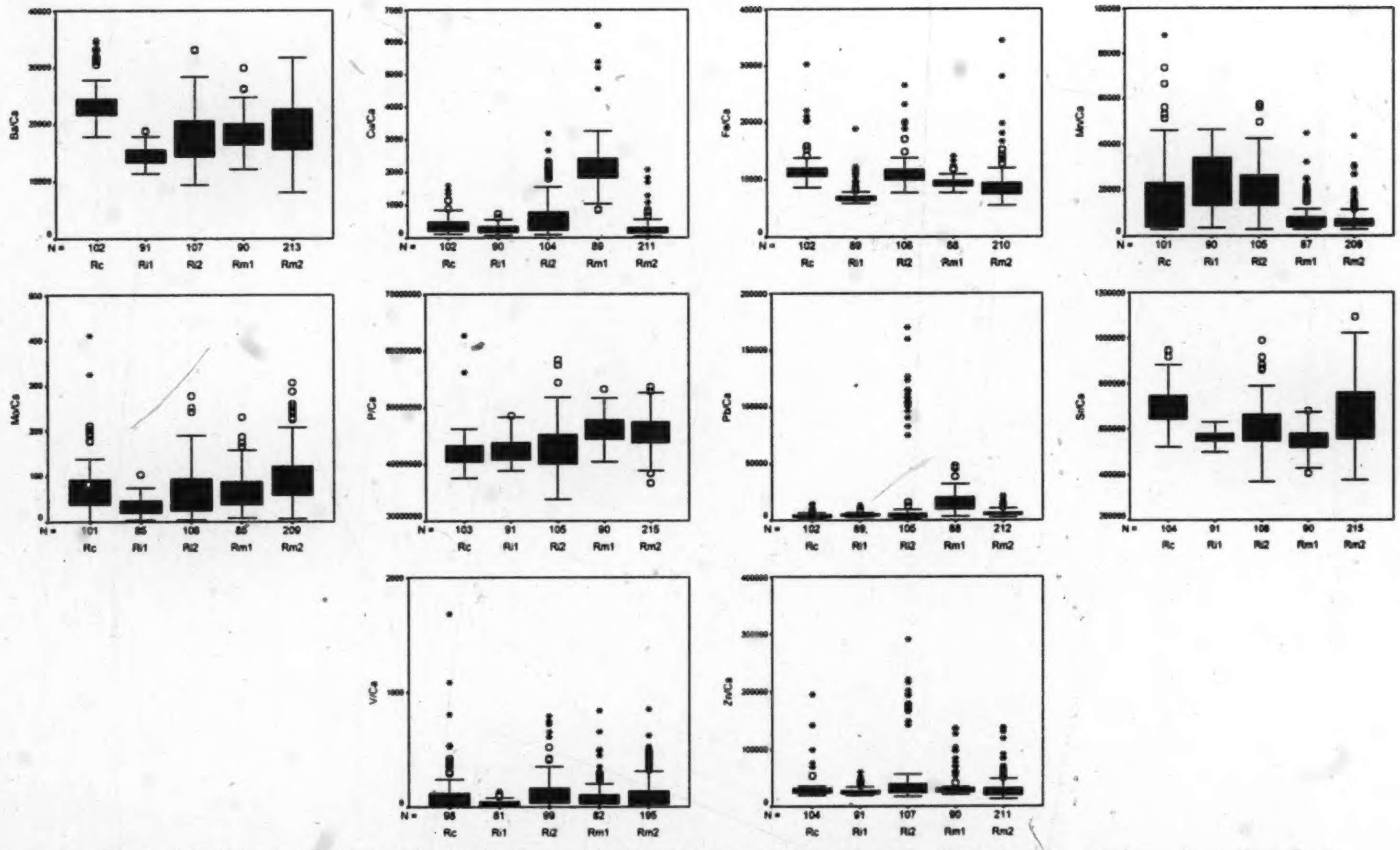

*Fig. 5.26a: Boxplots for individual B, showing the distributions of the Ca ratios for each tooth separately. Along the x-axis are shown the number of points measured on each line (N) and the teeth. Rc = right canine; Ri1 = right first incisor; Ri2 = right second incisor; Rm1 = right first molar; Rm2 = right second molar. ○= outlier, *=extreme value.*

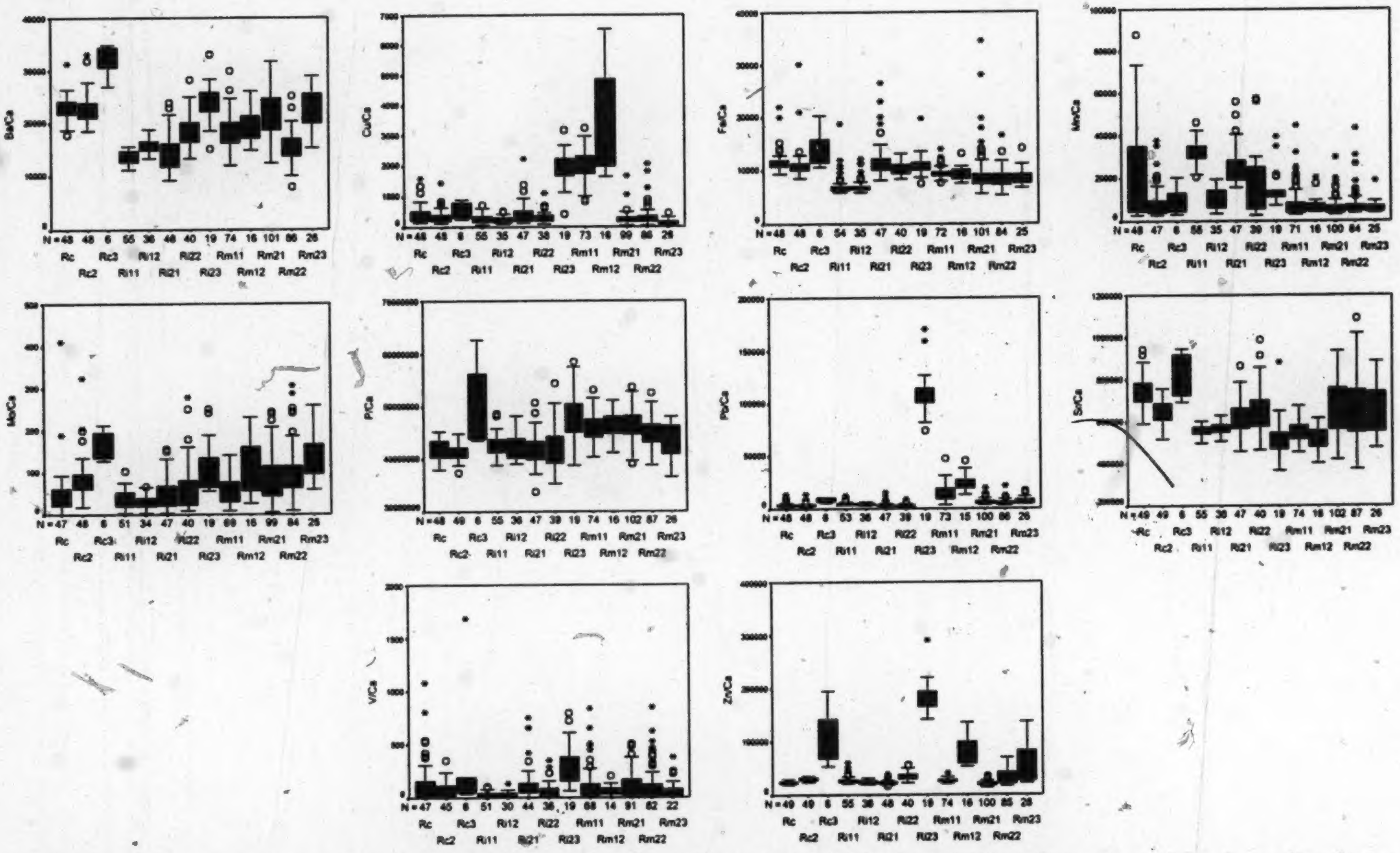

*Fig. 5.26b: Boxplots for individual B, showing the distributions of the Ca ratios for individual lines separately. Along the x-axis are shown the number of points measured on each line (N), the teeth and the longitudinal lines on each tooth. Rc = right canine; Ri1 = right first incisor; Ri2 = right second incisor; Rm1 = right first molar; Rm2 = right second molar; Rc = right canine, line 1; Rc2 = right canine, line 2 etc. ○= outlier, *=extreme value.*

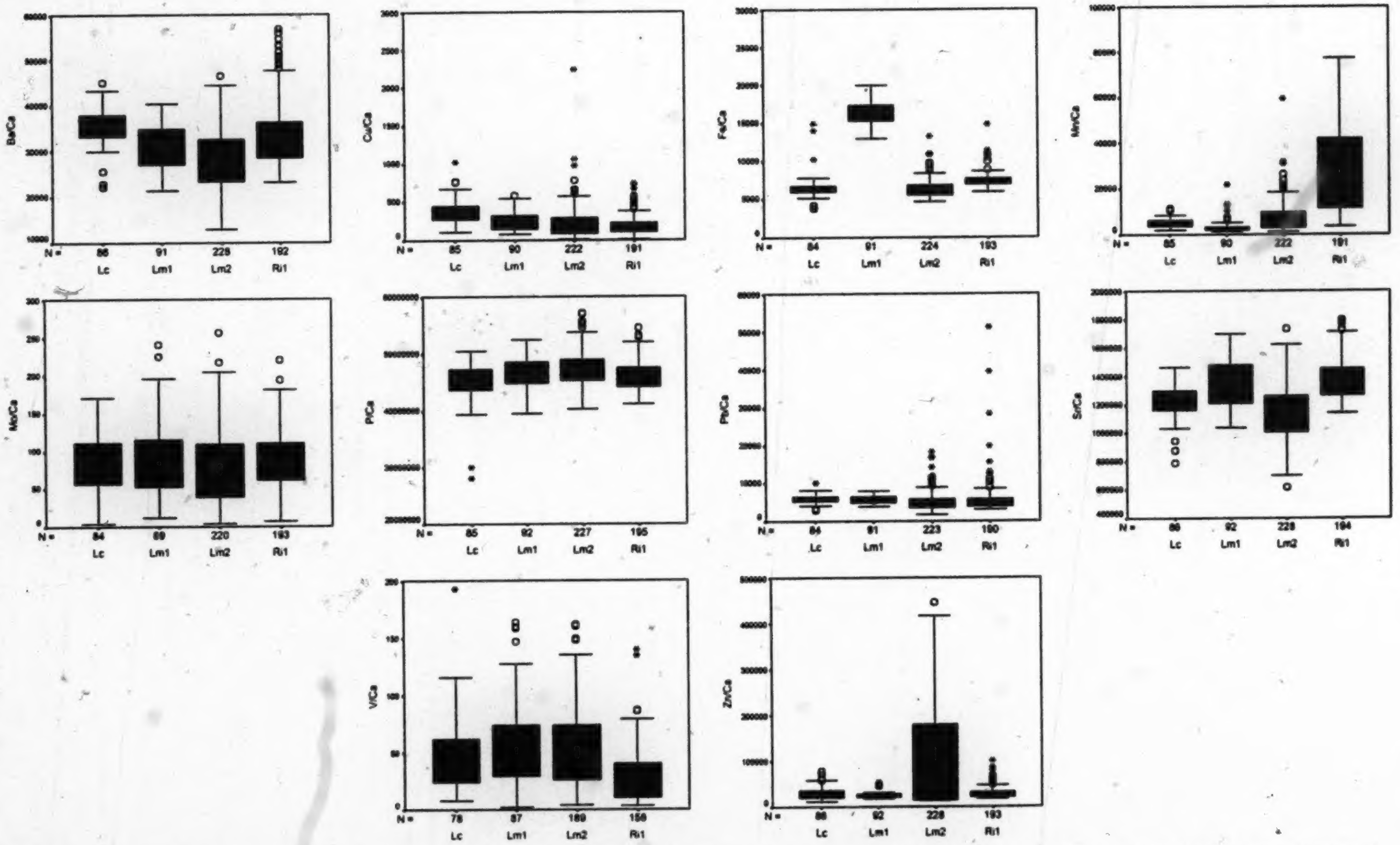

*Fig. 5.27a: Boxplots for individual C, showing the distributions of the Ca ratios for each tooth separately. Along the x-axis are shown the number of points measured on each line (N) and the teeth. Lc = left canine; Lm1 = left first molar; Lm2 = left second molar; Ri1 = right first incisor. ○= outlier, *=extreme value.*

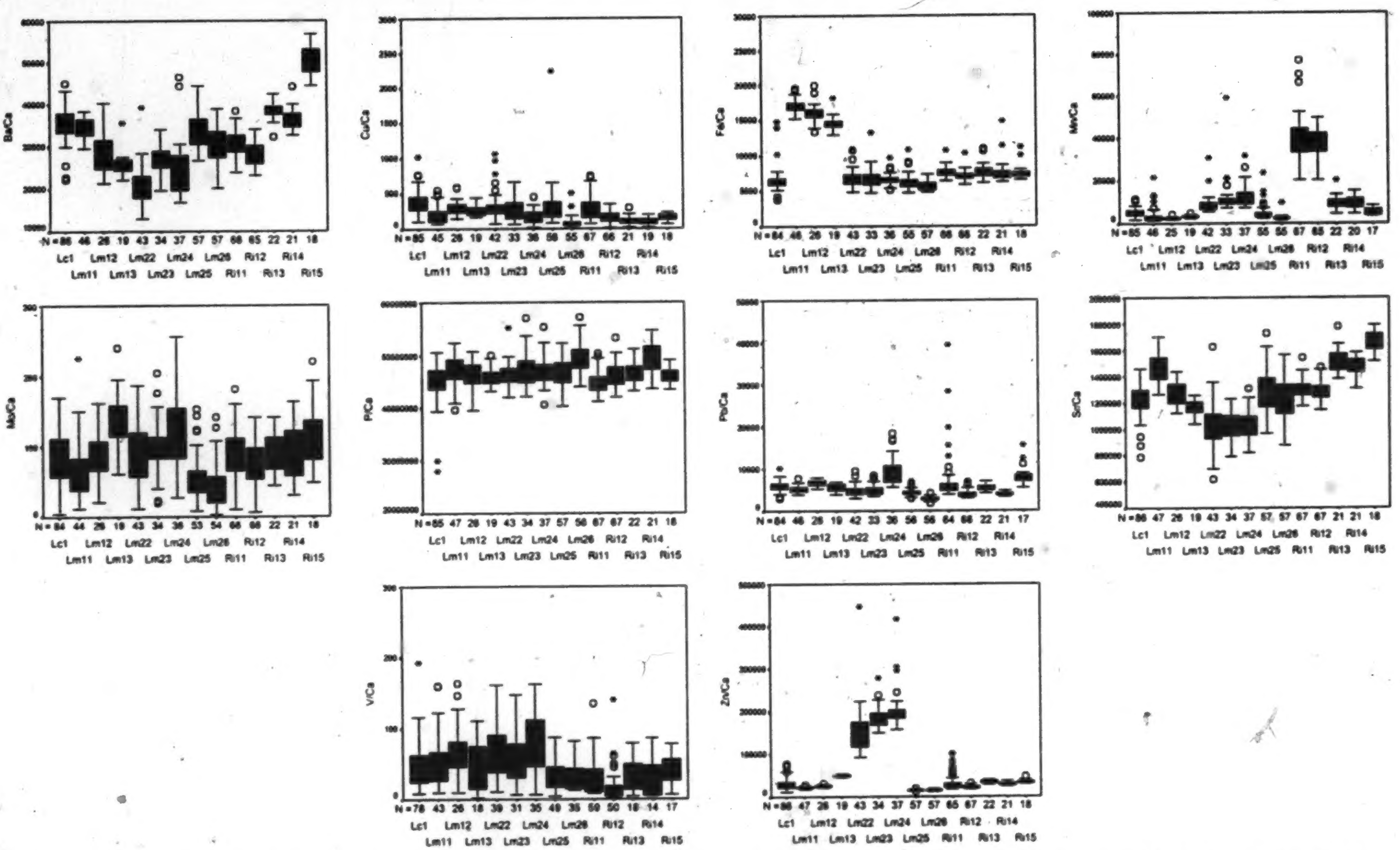

Fig. 5.27b: Boxplots for individual C, showing the distributions of the Ca ratios for individual lines separately. Along the x-axis are shown the number of points measured on each line (N), the teeth and the longitudinal lines on each tooth. Lc = left canine; Lm1 = left first molar; Lm2 = left second molar; Ri1 = right first incisor. Lc1 = left canine, line 1, etc. ○= *outlier*, *=*extreme value.*

Differences between individuals – a comparison of results with predictions

To get an impression of how different the teeth from all the individuals are from each other, average elemental concentrations were calculated for all the longitudinal lines. As mentioned before, averages are imperfect indicators of central tendency for the non-Gaussian distributed elemental concentrations in teeth, but they can serve as preliminary indicators of inter-individual differences.

For each element the average of each longitudinal line was plotted, grouped by individual (Fig. 5.28). These plots (as did the box plots) show that there is substantial variability within a single individual. Strontium, and to some extent Ba, is the only element that shows a more distinct clustering of values within each individual, and a separation between individuals. This is shown in the bi-plot of Sr and Ba (Fig. 5.29). The bi-plot also demonstrates the approximately linear relationship between Ba and Sr values. This is expected, based on the similarities in their chemical behaviour.

The bi-plot shows that the highest Ba and Sr values are found for individuals A and C. These two individuals were predicted (according to the model) to have higher concentrations of these elements in their enamel based on their consumption of formula, as opposed to the other individuals who were breastfed. The predictions based on the palaeodietary method did not distinguish between the individuals A, B and C with regard to Sr levels. Teeth from A and C were predicted to have relatively high Sr concentrations based on their mother's poor, probably vegetable-based, diet. On the other hand, teeth from B were predicted to have relatively high Sr levels based on maternal intake of marine food sources.

The Sr/Ca ratio was predicted to show changes associated with the introduction of solid foods. This change was expected to occur slightly earlier for individuals B and C compared to A. Given the developmental periods of the deciduous teeth (Table 2.2), a change occurring around 3-6 months could be recorded in the canine and both molars. A change occurring around 6-7 months could be visible in either the canine or the second molar. However, Sr appears to be relatively stable along the longitudinal lines, and the box plots also suggest that there are no significant differences between the different teeth for each individual. Therefore, the current dataset does not reveal changes in Sr/Ca ratio associated with the introduction of solid foods.

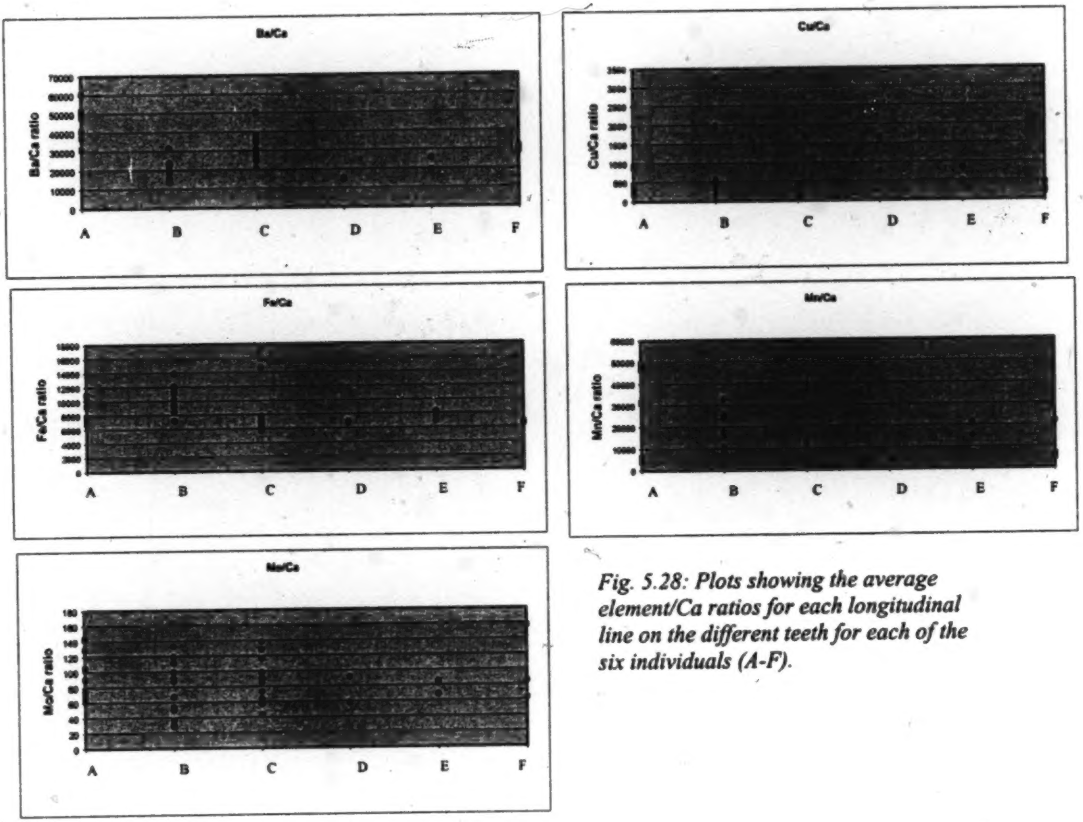

Fig. 5.28: Plots showing the average element/Ca ratios for each longitudinal line on the different teeth for each of the six individuals (A-F).

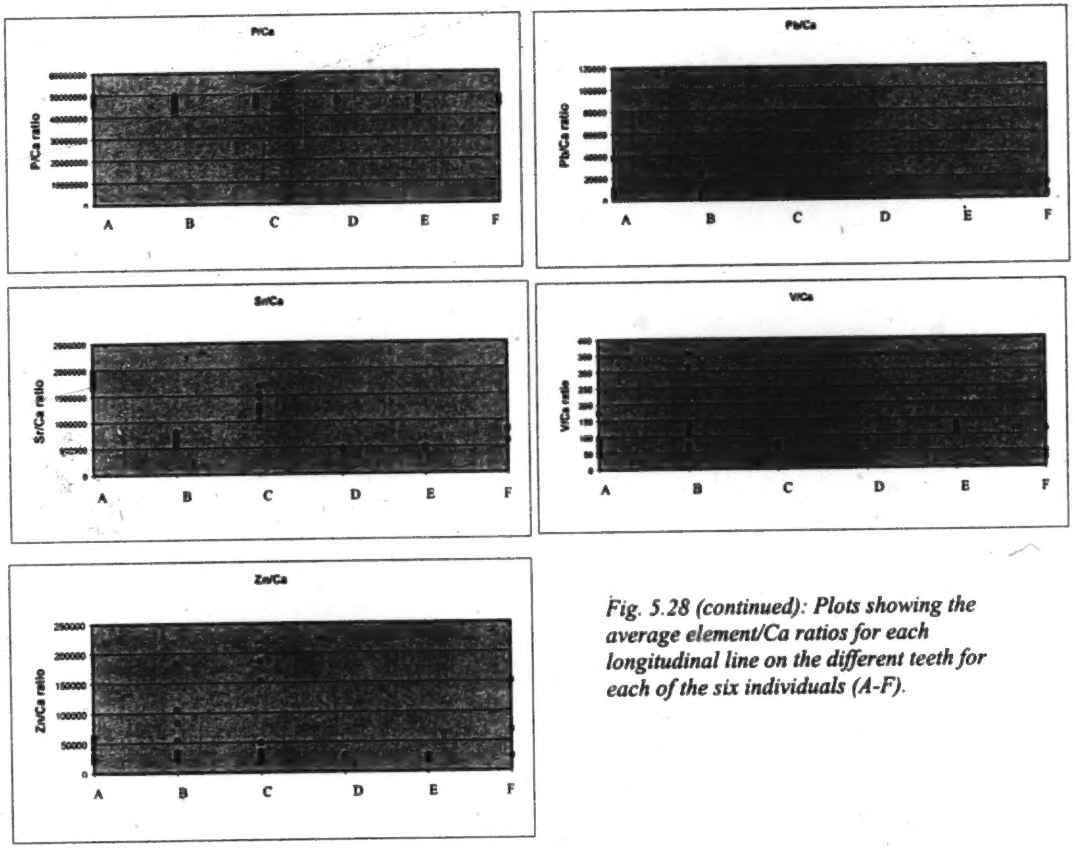

Fig. 5.28 (continued): Plots showing the average element/Ca ratios for each longitudinal line on the different teeth for each of the six individuals (A-F).

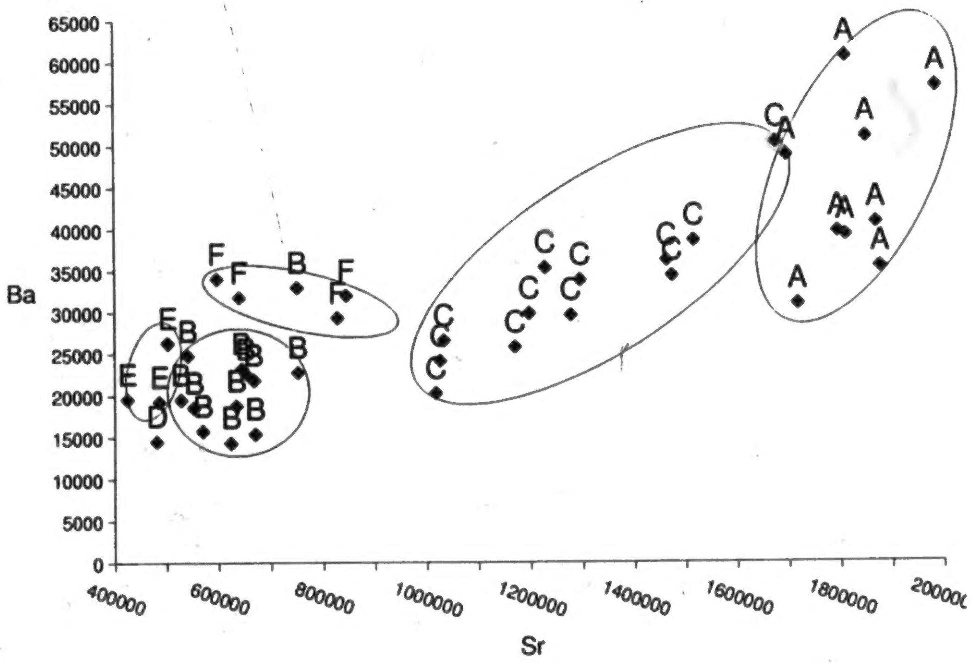

Fig. 5.29: Bi-plot showing the average Sr/Ca and Ba/Ca ratios calculated for each longitudinal line on the teeth from the six individuals (A-F) included in this study.

Individual D was born in a different geographical region than his sibling (ind. E) which could have resulted in differences in Sr intake from drinking water. However, the Sr values in their teeth are actually very similar (Fig. 5.29), which could indicate that the Sr levels in the drinking water of Hamilton and Edmonton are sufficiently similar so that differences are not detectable, or possibly that Sr concentrations in breast milk are characteristic for the mother, resulting in a similar intake of this element for both siblings.

Copper and Zn were predicted to be lower in teeth from A and C according to the palaeodietary method. The higher Zn values in some of the lines for B and C can be explained by the inclusion of more material from outer enamel layers that are enriched in Zn. Once these values are eliminated, most of the Zn values for all the individuals are fairly comparable. Copper concentrations are also very similar among the individuals. Three lines for individual B form the exception: line 3 on B-Ri2 and lines 1 and 2 on B-Rm1. The first one can be attributed to the effect of slightly higher Cu values in the outer enamel layer (see plot of longitudinal line – Fig. 5.23b). This line is also situated on the edge of enamel/epoxy. Line 2 on B-Rm1 shows an increase towards the edge of enamel, but otherwise the Cu concentrations in this molar do appear to be higher throughout the crown. Given the overlap in developmental time for all deciduous teeth it is difficult to explain the consistently higher Cu concentration in the second molar in terms of Cu intake during development.

The Mn concentration is substantially lower in the second molar (A-Rm2) compared to the other teeth of individual A (see box plots). These low values were measured along four different lines, covering both outer and inner enamel regions. As with Cu, it is difficult to explain this pattern based on diet during development.

Individual C shows high Fe values in the first molar (C-Lm1). The plots for the longitudinal lines show that the Fe levels in this tooth are higher for the whole trajectory and not just a particular region. Again, this result is very difficult to interpret in terms of diet.

Individual A was taking iron supplements between 3 and 7 months after birth to treat a low level of haemoglobin, but her enamel does not show higher Fe concentrations. Whereas the palaeodietary method can only predict a higher Fe concentration in enamel based on this higher intake, the model allows for the possibility that Fe transfer from mother to fetus is suboptimal, which may result in lower Fe stores in the neonate and development of anaemia (which indeed happened). The plots for the longitudinal lines show the remarkably constant levels of Fe along the length of every tooth. In addition, the box plots show that, if there is a difference in Fe concentrations between the teeth, Fe levels are somewhat higher in the incisors. According to Table 2.2, these teeth had completed their mineralization when the iron supplements were started at about 3 months after birth. The moment of changing Fe intake would have taken place during the mineralization of the canine (which was not available for analysis) and both molars. However, no such changes are visible in the plots, which suggests that the change in Fe intake, if indeed recorded in enamel, cannot be detected with current data quality.

CHAPTER 6

SUGGESTIONS FOR FUTURE STUDIES AND CONCLUSION

Suggestions for future studies

Laser ablation ICP-MS is a relatively new technique. Its use for the measurement of intra-tooth variation in trace element composition of enamel is promising, but the most suitable methodology for this application has not yet been determined. Below, some of the problem areas and potential solutions are presented to guide future research.

‘Memory effect’

An important current problem is that localized compositional changes, or cracks, affect the signal for a distance far beyond the localized feature. This effect reduces the spatial resolution and makes it more difficult to analyze boundary effects and to distinguish between artefacts and true signals. The memory effect results from the transport of the ablated material through the tube system from the sample chamber to the detector. This causes a delay and allows material from subsequent laser pulses to mix. The effect of this process on the data can be minimized by reducing the scan speed of the laser. This will reduce the distance travelled by the laser during the ‘memory time’, thereby improving spatial resolution. The data collection time will increase in this way, but since a large proportion of the time is spent on handling of the sample and defining the location of the laser tracks, the effect on the total data collection time will be minor.

Beam width

In choosing the optimal diameter of the laser beam for a particular application, one has to compromise between spatial resolution and data quality. Larger beams give higher counts and thus more accurate data. Since we anticipated gradual composition changes along the time axis of a tooth crown, spatial resolution was not a high priority. However, much sharper concentration gradients exist in the cross-sectional direction from EDJ to the enamel surface. In retrospect, a smaller beam diameter would have been desirable, especially for the anterior deciduous teeth with thin enamel. To obtain sufficient counts, *i.e.* to maintain data quality, a smaller beam diameter should be combined with a slower scan speed and/or the analysis of fewer elements (for example Ca, Ba, Sr, Fe, Mn, Zn). A slower scan speed has the added benefit of reducing the memory effect (see above).

Spot analysis

For samples with extreme cracking (e.g., many archaeological samples), continuous line scans may no longer be feasible. In this case one could consider measuring spots along a line following the curvature of the EDJ in such a way that cracks are avoided.

Pre-ablation

Although the sample needs minimal preparation for laser ablation analysis, there is always the possibility of surface contamination during handling of the sample. To avoid the effects of such contamination one could pre-ablate the laser track prior to the actual analysis. During the pre-ablation the laser is run at higher speed along the predefined track to ablate the potentially contaminated top layer of the sample. However, during this study, we analyzed the same trajectory on the second molar of ind. C two times (lines 5 and 6). It was found that the counts for all elements were reduced during the second analysis. The reason for this is not immediately apparent, and it indicates the importance of studying the effects of pre-ablation prior to any use of this method of sample preparation.

SEM-imaging

The SEM images suggested that some of the cracks in the samples are induced by the laser during the analysis. To confirm this, SEM images of the sample should be prepared prior to, as well as after, the analysis. An SEM image of the sample prior to analysis can be used as a map on which cracks and other surface features can be marked. During the analysis, this map can assist with selection of areas for spot analysis or with determining where to position linear tracks. In addition, SEM can be used to study the morphology of the tracks created by the laser and the variability of the ablation volume. Conventional SEM requires carbon coating of samples to prevent charging. Not only would this introduce a surface contamination, the coating can obscure surface features when viewing the sample during laser ablation analysis. It is therefore preferable to use a low voltage SEM, or even an environmental SEM, which allow viewing of samples without coating.

Sample preparation

Teeth should be sectioned in a standardized way, to minimize the number of variables that can contribute to variability in composition (see p. 140). The sectioning plane should be as straight as possible, going through the highest point of the molar cusps or incisal edge. In addition, it would be very useful to have thin sections for histological analysis to be carried out in conjunction with the chemical analysis. The sample preparation method used in this study (Fig. 6.1), is appropriate for this purpose. Cutting the tooth at line A yields a section (I) that can be used for laser ablation analysis. A second cut at line B yields a section (II) that can be further prepared for histological analysis. Because the sectioning blade has a certain thickness, a thin layer of the tooth is lost from between parts I and II. Therefore, the surface analyzed with laser ablation ICP-MS and the thin section will not reflect exactly the same area of the crown, but they will be close enough to allow a comparison of shifts in chemical composition with histological features.

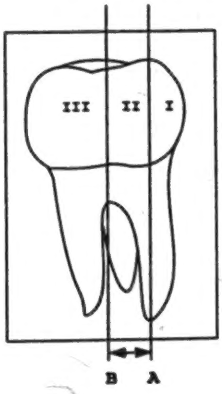

Fig. 6.1: The sample preparation method used in this study can be used for future studies including LA-ICP-MS and histological analysis of the same specimens. The tooth is cut at line A and line B. The second cut can be made in such a way that a thin section can be prepared from section II, while section I can be used for laser ablation analysis. Section III can be stored for possible future applications.

Histological analysis of the incremental structures (Retzius lines and microstriations) of a tooth can be used to provide an internal time line for the development of that tooth. The neonatal line, if present, can be used to calibrate tooth development relative to time of birth. The compositional data obtained can then be superimposed on this developmental time axis. Combined with the dietary records, this may provide insights into the nature of the mineralization process. The histologically derived time line is also necessary to determine if features in the elemental profiles from lines in different teeth correspond to the same period of development. This could, for example, be used in a further analysis of the Pb patterns that were found in this study in several of the permanent teeth.

Samples

At this stage, samples with known history are absolutely essential to unravel the relations between diet and enamel composition. We do not yet know to what extent levels of trace elements in the diet are reflected in enamel concentrations. Neither do we know how large a difference in trace elemental intake is required to be detectable in enamel. The unique collection of teeth from individuals with known dietary history, which has been explored in this study, will be available for future studies. In addition, teeth from other individuals who experienced major changes in diet during the period of dental development are highly desirable.

Conclusion

Laser ablation analysis is capable of detecting intra- and inter-tooth variation in trace element concentrations in dental enamel. Data collection is fast and convenient and because of the sensitivity of the technique many trace elements can be included in the research design. The samples need minimal preparation so that the risk of contamination is significantly reduced. Among the disadvantages are the fact that the technique is not yet widely available, and the lack of suitable standards. The latter means that results are semi-quantitative only and cannot directly be compared with those reported in the literature.

The data show that there is substantial intra-tooth variation. Based on the SEM images some of this variation can be explained as artefacts due to cracks or edge effects. Furthermore, the beam diameter employed in this laser ablation study posed more serious problems for the deciduous samples, which makes a full evaluation of these data difficult. However, the analysis of the permanent teeth showed that there is variation for some trace elements between teeth and within a tooth that must have another, potentially diet related, cause.

The variation in elemental concentrations that is observed along the length of the crown and across the width of enamel indicate that, when small samples are taken from a tooth, both sampling location on the crown, and sampling depth, will affect the results.

This is clearly demonstrated by the NAA results where the composition of the two central sections of the molar differs from that of the two outer sections.
Because the concentrations of some elements show a gradual increase in the outer layers with increasing age, the age of the individuals from which tooth samples derive must also be taken into account. For cross-population comparisons the age distribution should therefore be comparable. Another factor that plays a role in this case is the degree of wear. Elements that are concentrated mainly in the surface layers, such as Zn and Pb, will be affected by patterns of wear – not only on the occlusal surface but also on the sides of the teeth (M. Jackes, *pers. comm.*).

The differences between the different teeth of each individual were difficult to assess in this study, given the interfering effects of the beam diameter in relation to the width of enamel on especially the anterior deciduous teeth. Differences between individuals were not clearly related to dietary records and the expectations put forward in Chapter 4. However, based on the model developed in Chapter 3 and technical considerations it appears that in future studies it is better to focus on a smaller number of trace elements. The elements Ba, Sr, Fe, Cu, Mn and Zn are of most interest from a dietary perspective. As was stated in Chapter 1, one of the reasons for studying infant nutrition is that dietary deficiencies of some trace elements (e.g., Fe, Cu and Zn) can have a crucial effect on infant health and development (and thus, on infant survival and population dynamics). These elements can be measured reliably with LA- ICP-MS.

Another issue of importance concerns the nature of the mineralization process, which was discussed in Chapter 2. Generally speaking, none of the 2-D maps show a shift in elemental concentrations along the axis of crown development. If there are differences in concentration, these usually take the shape of 'zones' from outer to inner enamel, along the EDJ, or along the innermost dentine lining the pulp cavity. This zonation in fact is similar to Suga's description of the different zones that he observed during maturation of enamel in the teeth of several species (Suga, 1982, 1983, 1989). This may indicate that the volume of the crown is fully formed before the major increase in mineralization takes place, resulting in a more even distribution of the chemical constituents across the crown than is predicted by the model of gradual mineralization during crown formation (see Chapter 2).

The results obtained show that there is significant variation within and between teeth. However, even with the dietary records, interpretation of the observations is not straightforward, by either the palaeodietary method, or the model developed in this thesis. At this stage, therefore, more detailed evaluation of the relative merits of the methods is not possible. Nonetheless, the model – although still in a very preliminary form – incorporates various biological principles and has therefore inherently more potential to predict elemental levels correctly.

One problem is that both models predict the *direction*, as either an increase or decrease, of changes in chemical composition, but they do not give insight into the *magnitude* of the change. Based on the various 'barriers' and homeostatic regulation systems described in the model proposed here, it can be expected that changes in enamel composition will not be proportional to changes in dietary intake for some elements. In extreme cases, the concentrations in enamel may be independent of dietary intake when dietary levels are within a range corresponding to adequate levels.

One important goal is to determine which elements do reflect dietary intake. Strontium and Ba are generally considered valid dietary indicators in bone studies, given their relation to Ca in metabolism, and the lack of homeostatic control mechanisms for these elements. Zinc is also considered useful and often included in studies (but see Ezzo 1994a). Our study further suggests that Cl, Mn, Fe and Cu show variability that is worth consideration, although a relation with dietary intake was not immediately clear.

With improvements to existing analytical techniques, and with the development of a proper methodology for studying enamel composition on a microscale, it may be possible to detect increasingly smaller concentration differences within a tooth. However, as was shown in this thesis, it is important to be aware of how sampling size and location can affect the measurements, and thereby the interpretation of the elemental concentrations in terms of diet. It is essential first to develop the methodology on samples of known history to validate the suitability of, for example, laser ablation analysis, and to optimize the methodology prior to application of this technique to archaeological samples.

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APPENDICES

APPENDIX A: Periodic Table of the Elements

The elements selected for analysis with Laser Ablation ICP-MS are marked in grey.

Group	1A	2A	3B	4B	5B	6B	7B	8X	8Y	8Z	1B	2B	3A	4A	5A	6A	7A	8A
Period																		
1	1 H																	2 He
2	3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
3	11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
4	19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
5	37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
6	55 Cs	56 Ba	LA	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
7	87 Fr	88 Ra	AC															

Ag	*Silver*	He	*Helium*	Rb	*Rubidium*
Al	*Aluminum*	Hf	*Hafnium*	Re	*Rhenium*
Ar	*Argon*	Hg	*Mercury*	Rh	*Rhodium*
As	*Arsenic*	I	*Iodine*	Rn	*Radon*
At	*Astatine*	In	*Indium*	Ru	*Ruthenium*
Au	*Gold*	Ir	*Iridium*	S	*Sulphur*
B	*Boron*	K	*Potassium*	Sb	*Antimony*
Ba	*Barium*	Kr	*Krypton*	Sc	*Scandium*
Be	*Beryllium*	Li	*Lithium*	Se	*Selenium*
Bi	*Bismuth*	Mg	*Magnesium*	Si	*Silicon*
Br	*Bromine*	Mn	*Manganese*	Sn	*Tin*
C	*Carbon*	Mo	*Molybdenum*	Sr	*Strontium*
Ca	*Calcium*	N	*Nitrogen*	Ta	*Tantalum*
Cd	*Cadmium*	Na	*Sodium*	Tc	*Technetium*
Cl	*Chlorine*	Nb	*Niobium*	Te	*Tellurium*
Co	*Cobalt*	Ne	*Neon*	Ti	*Titanium*
Cr	*Chromium*	Ni	*Nickel*	Tl	*Thallium*
Cs	*Caesium*	O	*Oxygen*	V	*Vanadium*
Cu	*Copper*	Os	*Osmium*	W	*Tungsten*
F	*Fluorine*	P	*Phosphorus*	Xe	*Xenon*
Fe	*Iron*	Pb	*Lead*	Y	*Yttrium*
Fr	*Francium*	Pd	*Palladium*	Zn	*Zinc*
Ga	*Gallium*	Po	*Polonium*	Zr	*Zirconium*
Ge	*Germanium*	Pt	*Platinum*		
H	*Hydrogen*	Ra	*Radium*		

APPENDIX B: Trace Elements and Food Sources

Table B-1: Overview of the most important food sources for each of the elements selected for laser ablation ICP-MS. The table lists which foods have either high or low concentrations of the elements used in this study.

Element	Selected food sources in rank order	Low (negligible sources)
Ba	Vegetable foods; wide variation in both animal and plant foods	Marine species
Ca	Cheeses (rich source); some nuts, fish with soft edible bones, green leafy vegetables, milk products, soybean flour, vegetables, bread	Meats, some vegetables, grains, cereals, fats and oils, most fish, most fresh fruits, mushrooms, peas, potatoes, tomatoes
Cu	Liver, oysters, Brazil nuts (rich sources); lobster, nuts and seeds, soybean flour, wheat bran and germ; beans, meats, breads and cereals, eggs, fish, green vegetables, banana, avocado	Fats and oils, milk and milk products, other fruits and vegetables
Fe	Organ meats, oysters, potato flour, rice polish, soybean flour, wheat bran and germ (rich sources); meats, clams, dried fruits, nuts, legumes, fish, green leafy vegetables, eggs	Fats and oils, cheese, fresh fruits, fruit juices and beverages, milk and milk products
Mn	Brown rice, rice bran and polish, walnuts, wheat bran and germ (rich sources); blueberries, lettuce, beans, soybeans, peanuts, sunflower seeds, wheat flower, whole grains, potatoes; most fruits and vegetables	Fats and oils, eggs, fish, meats, milk
Mo	Organ meats, whole grains, leafy vegetables, legumes NB: Concentration in plants depends on local soil levels (*i.e.*, geographic variation)	lowest in fruits, root vegetables, muscle meats and dairy products
P	Rice bran, rice polish, soybean flour, sunflower seeds wheat bran (rich sources); meat, liver, cheeses, fish and seafood, nuts, whole grains; breads and cereals, eggs, milk, most vegetables, mushrooms	Fats and oils, juices and beverages, some vegetables (lettuce, carrots, celery, tomatoes)
Pb	Contaminant	
Sr	Nuts (esp. Brazil nuts), legumes and leafy vegetables, cereals, whole grains (bran); molluscs, marine fish, shellfish; geographic variation [nb: drinking water]	maize
V	Shellfish, spinach, mushrooms, whole grains	(In general food contains very little)
Zn	Beef, liver, oysters, wheat bran (rich sources); crab, lamb, pork, poultry, peanuts; beans, clams, eggs, fish, wheat cereals, whole grains	Fats and oils, beverages, fruits and vegetables, milk

Sources: Ensminger et al. (1994); Groff et al. (1995); Mertz (1987) and the palaeodietary studies discussed in Chapter 1.

APPENDIX C: Electron probe micro analysis

Table C-1: *The measured elements, standards, x-rays and crystals used in the electron microprobe analysis. Results of the analysis are reported in weight% of the oxides. The oxide factor is used in the conversion of weight% of the oxides to the weight% of the elements. Detection limits are given in ppm. Accelerating voltage = 15.0 kV, probe current = 1.5E-08A (15 nA).*

Element	Oxide	Oxide factor	Standard	x-ray	Crystal	d.l. (ppm)
F	--	--	apatite	Kα	LDE1	241
Na	Na_2O	1.34797	kaersuitite	Kα	TAP	150
Mg	MgO	1.65809	osumilite	Kα	TAP	105
Al	Al_2O_3	1.88947	hypersthene	Kα	TAP	140
Si	SiO_2	2.13931	fayalite	Kα	TAP	121
P	P_2O_5	2.29137	apatite	Kα	PETJ	189
S	SO_3	2.49695	scapolite	Kα	PETJ	207
Cl	--	--	scapolite	Kα	PETJ	114
K	K_2O	1.20459	kaersuitite	Kα	PETJ	79
Ca	CaO	1.39919	apatite	Kα	LIFH	136
Fe	FeO	1.28649	obsidian	Kα	LIFH	197
Zn	ZnO	1.24475	rhodonite	Kα	LIFH	312
Ba	BaO	1.11649	sanidine	Lα	PETJ	467
Sr	SrO	1.18260	strontianite	Lα	TAP	219

Conversion of oxide weight% to ppm:

The results from the analysis have the form of oxide weight%. In order to transform these oxide % into ppm of element, they have to be converted, based on the following relationship between the two (oxide factors listed in Table A-1):

$$\{\text{atomic weight}_{(\text{element})} + \text{atomic weight}_{(\text{oxygen})}\}/\text{atomic weight}_{(\text{element})} = \text{oxide factor} \quad (1)$$

$$\text{weight\%}_{(\text{oxide})}/\text{oxide factor} = \text{weight\%}_{(\text{element})} \quad (2)$$

N.B.: 0.01% = 100 ppm

Table C-2: *Measurement order for the 14 elements.*

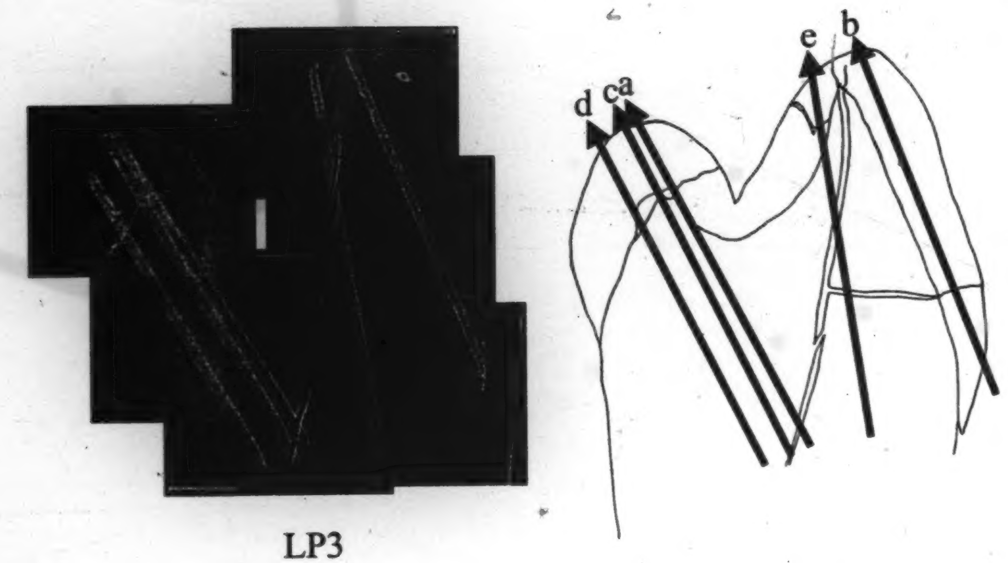
Order	Channel 1	Channel 2	Channel 3	Channel 4	Channel 5
1	F	Na	-	P	Ca
2	-	Mg	-	S	Fe
3	-	Al	-	Cl	Zn
4	-	Si	-	K	
5	-	Sr	-	Ba	

APPENDIX D: Laser Ablation ICP-MS (Permanent teeth)

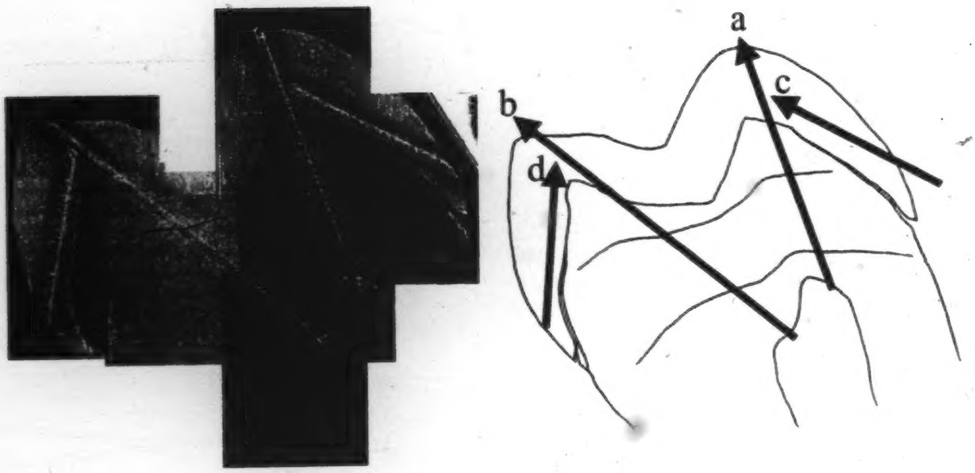
1. SEM-composites and line drawings based on these images, with arrows indicating the location and direction of the laser trajectories (pp. 224-226)

2. Plots showing the element and element/Ca ratios for the longitudinal lines on the permanent teeth. Figures D-1 to D-7 (pp. 227-233). **Cf. Figures 5.7-5.13.**

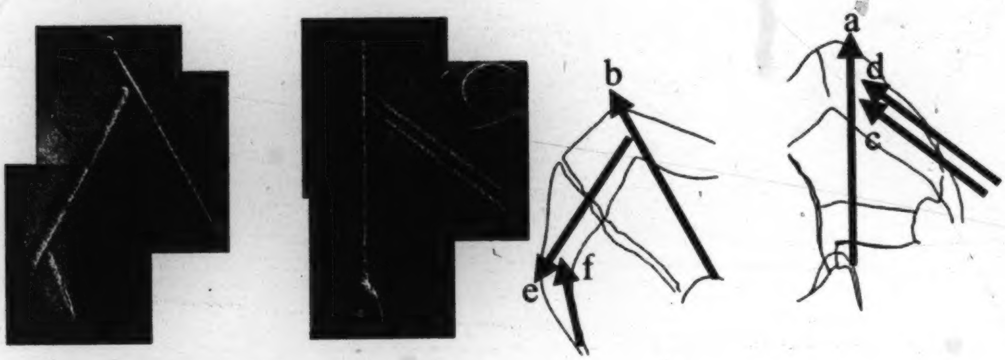
3. Summary graphs of laser tracks moving from inner dentine ('pulp') to outer enamel surface (edge) across the EDJ. Figures D-8 to D-13 (pp. 234-239). **Cf. Figure 5.18.**



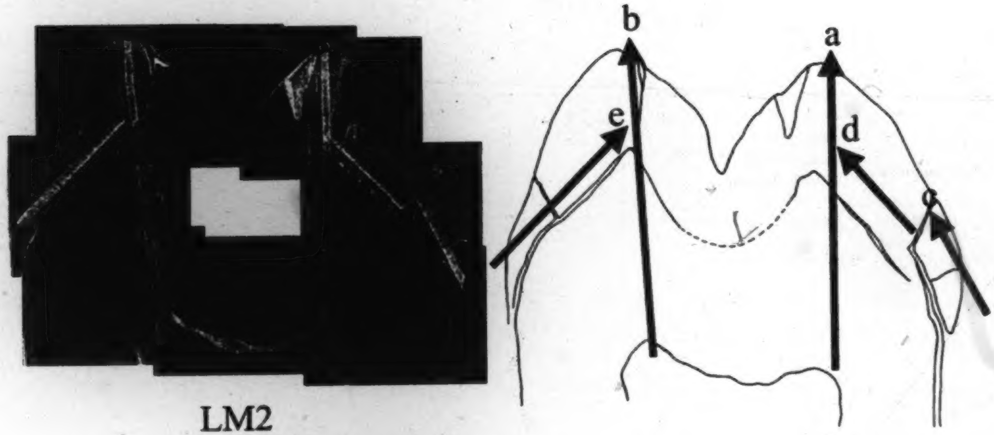
LP3



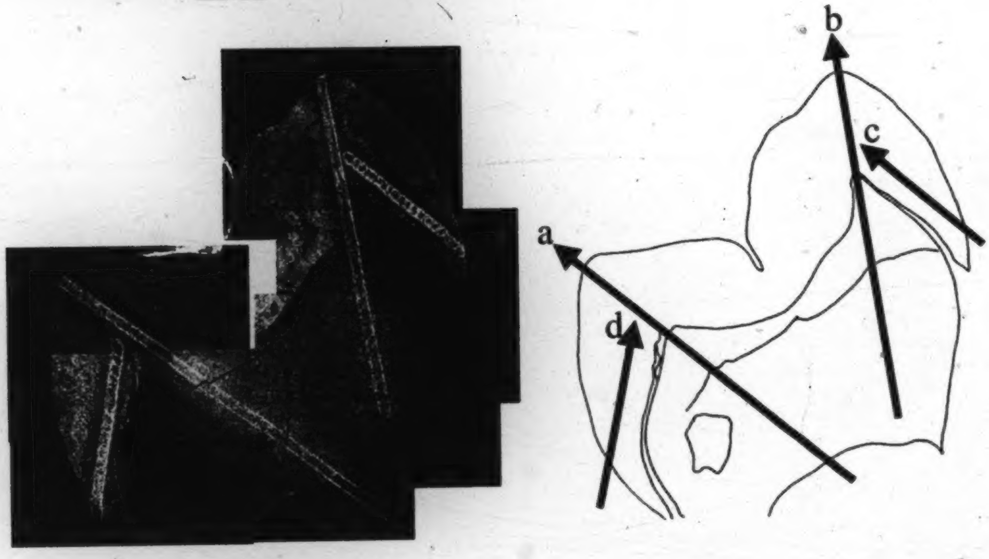
LP4



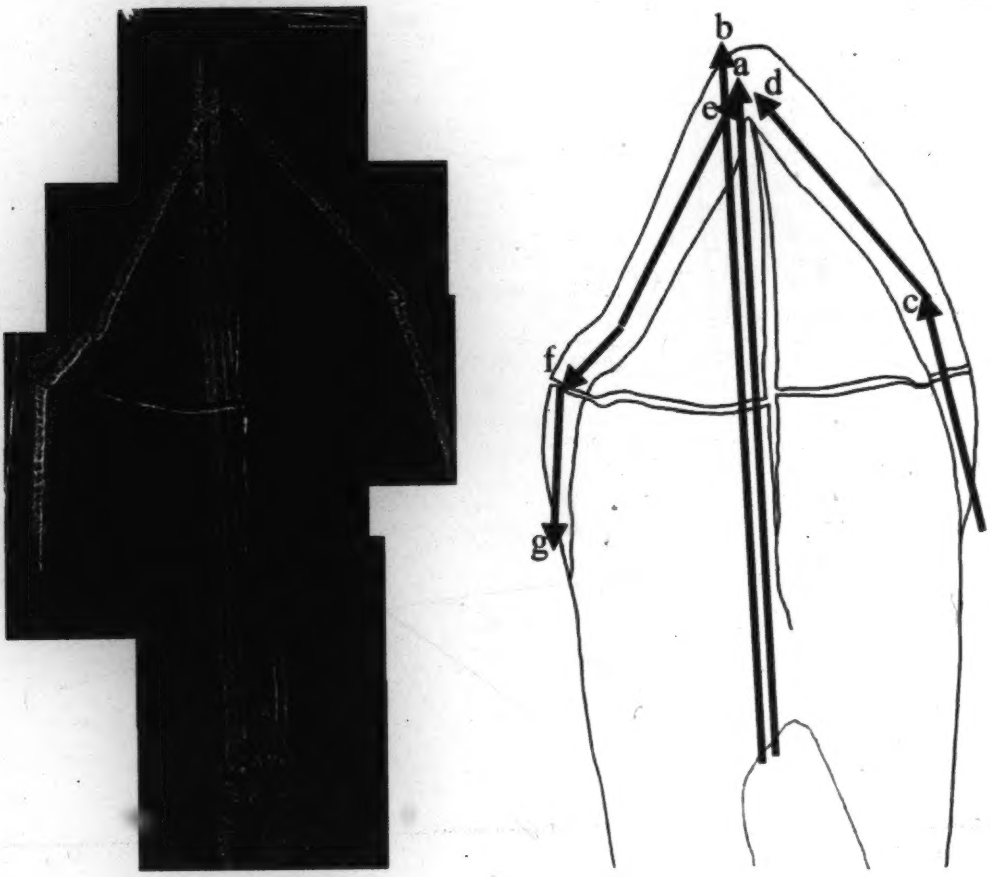
LM1



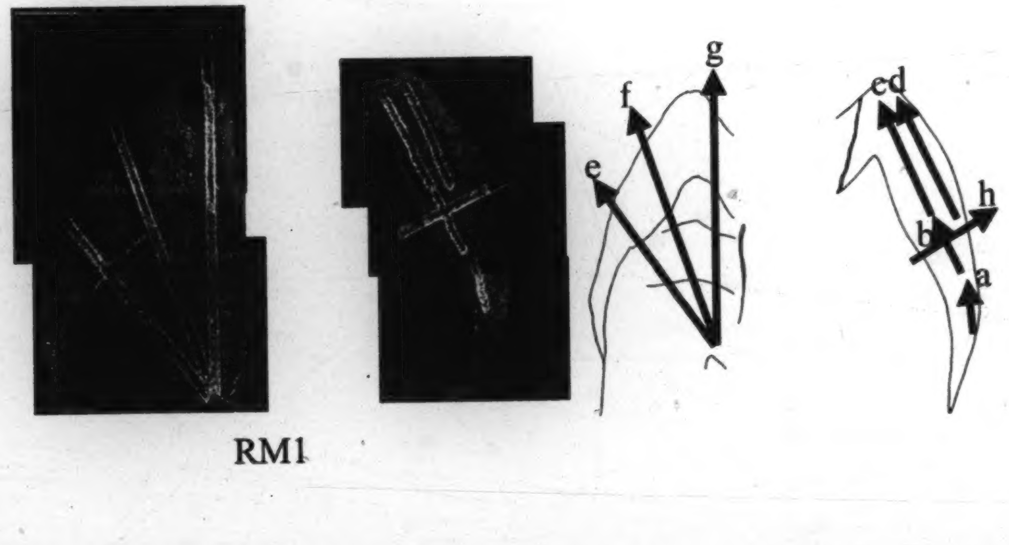
LM2



LM3



LC



RM1

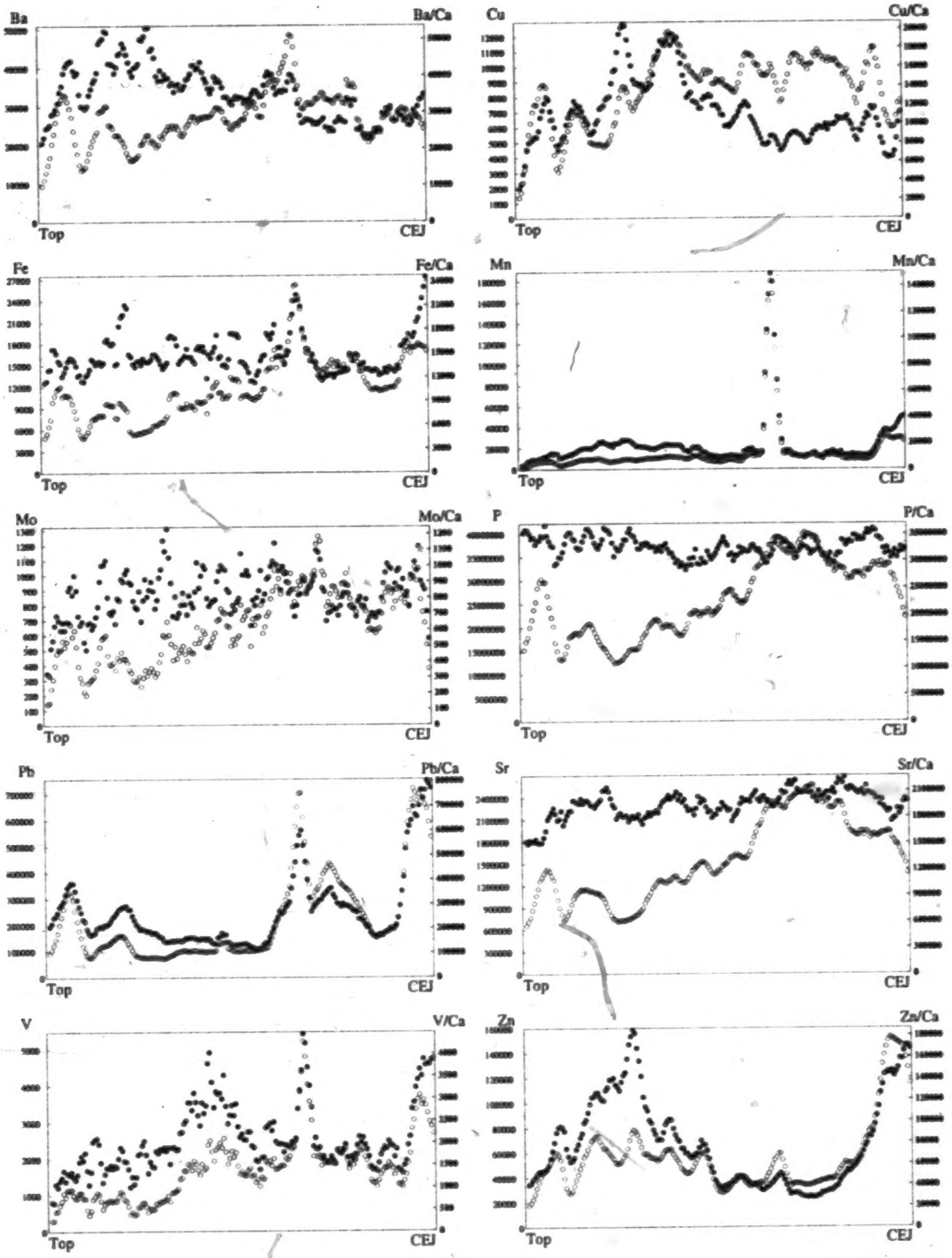


Fig. D-1: Element and element/Ca ratios for combined lines e, f and g on the left canine (P-LC) of the maxilla. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

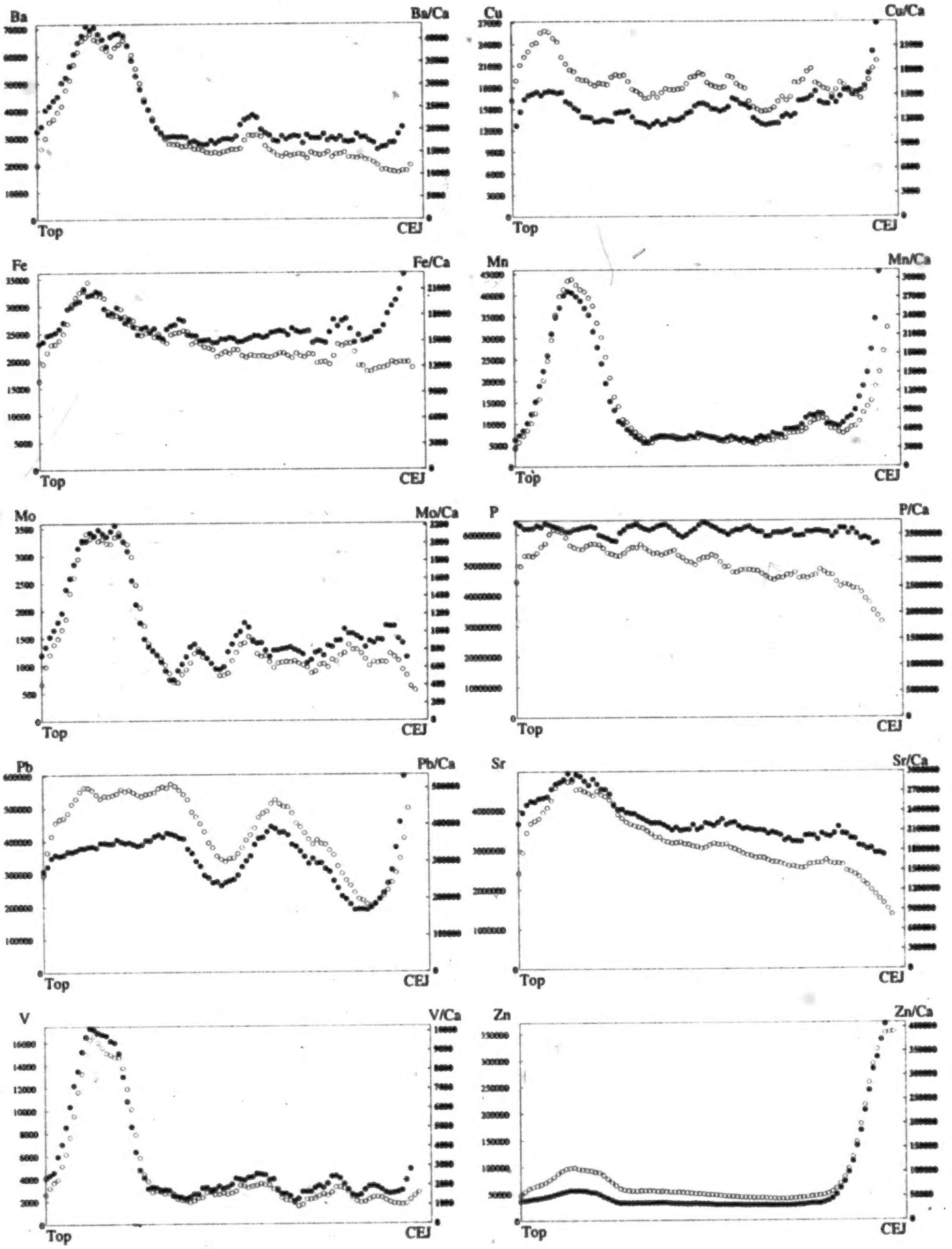


Fig. D-2: Element and element/Ca ratios for line c on the left second premolar (P-LP4) of the maxilla. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

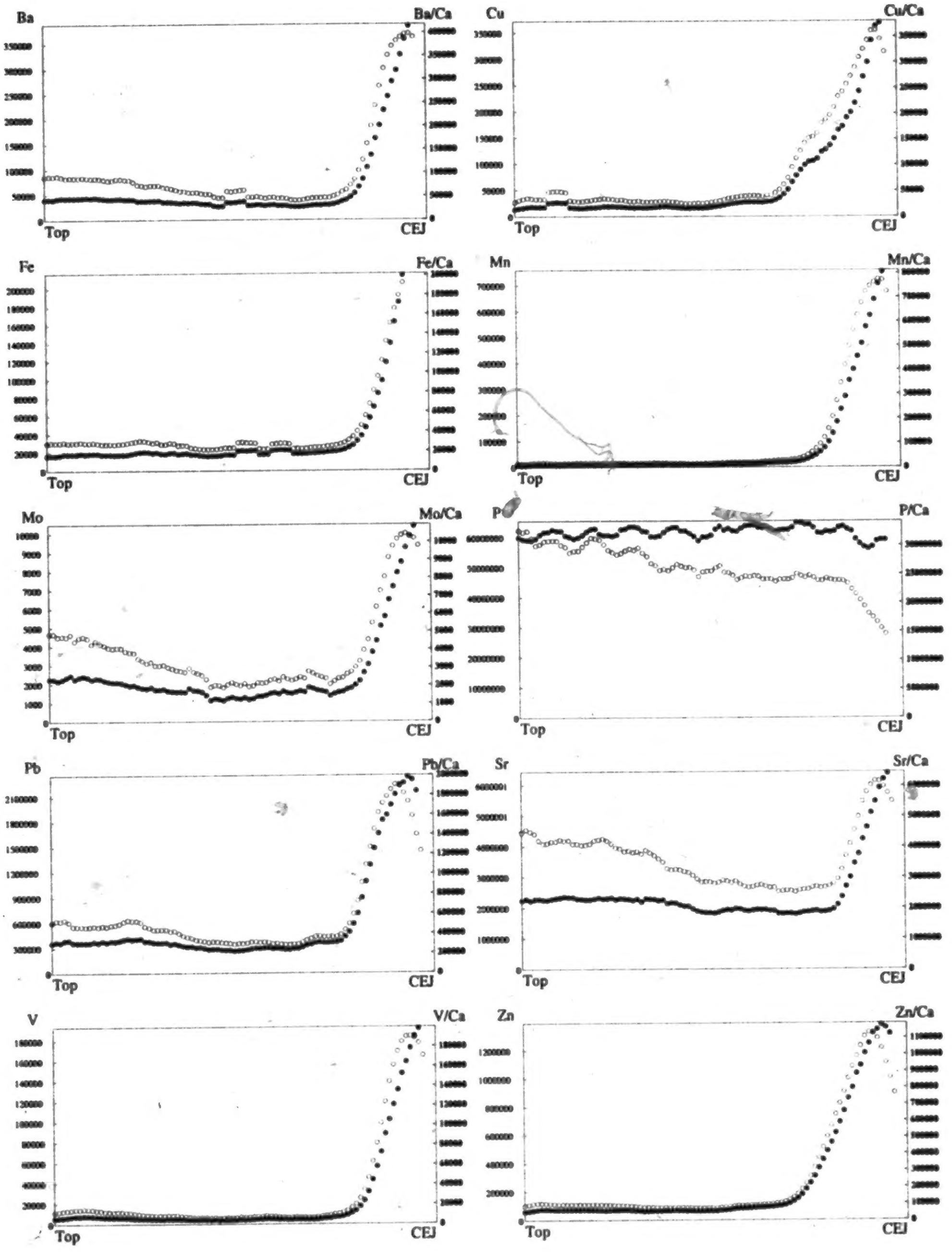


Fig. D-3: Element and element/Ca ratios for line d on the left first molar (P-LM1) of the maxilla. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

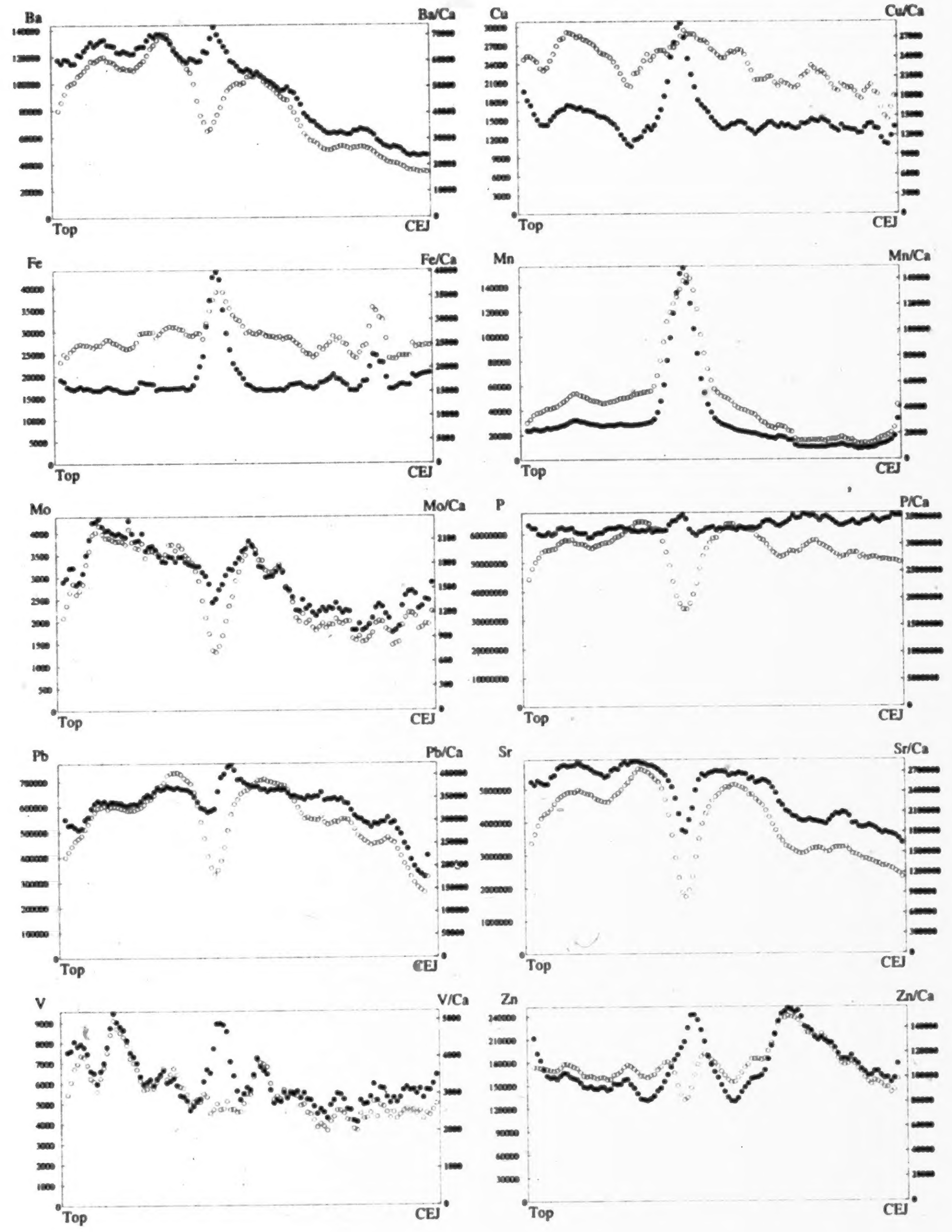


Fig. D-4: Element and element/Ca ratios for line e on the left first molar (P-LM1) of the maxilla. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

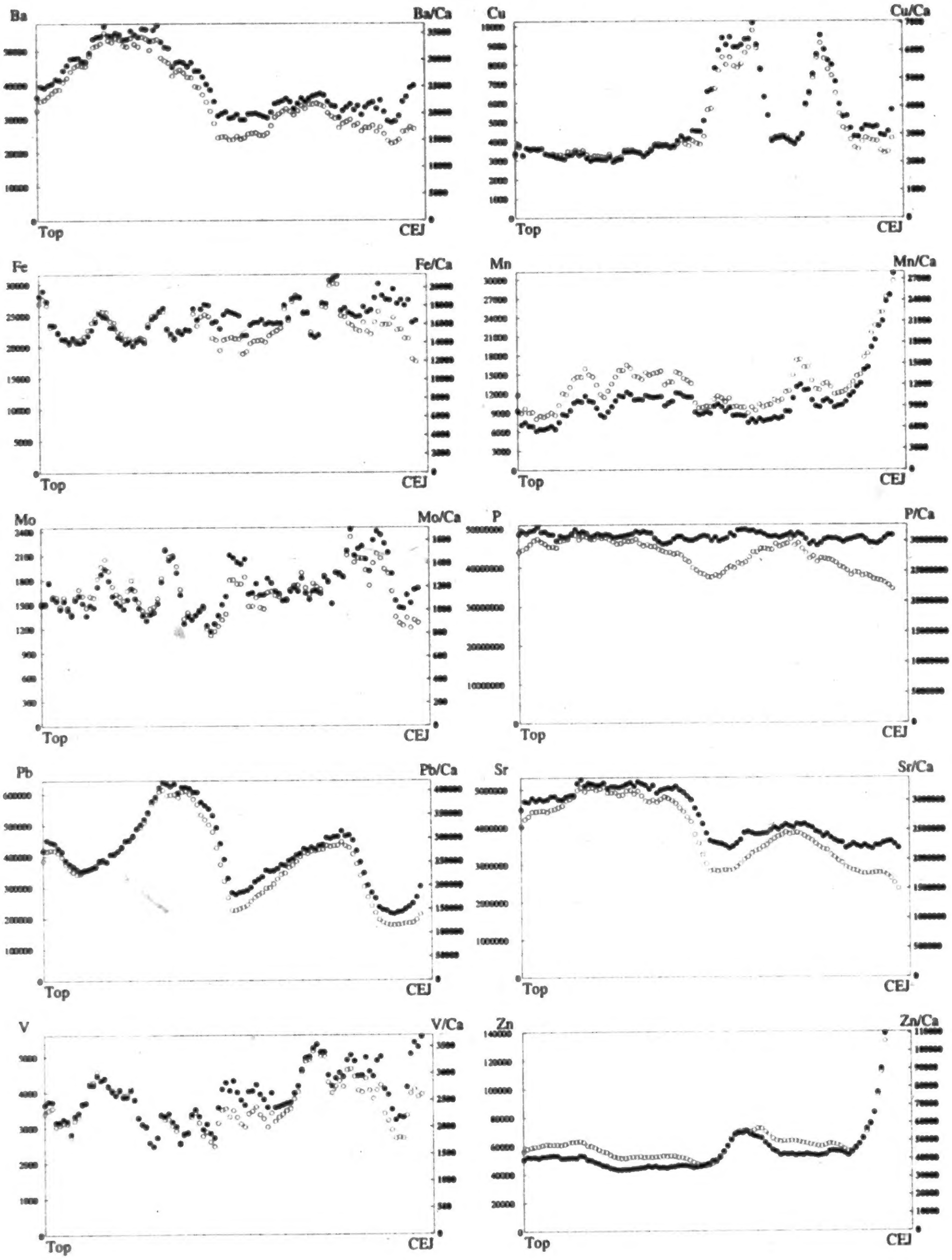


Fig. D-5: Element and element/Ca ratios for combined lines c and d on the left second molar (P-LM2) of the maxilla. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

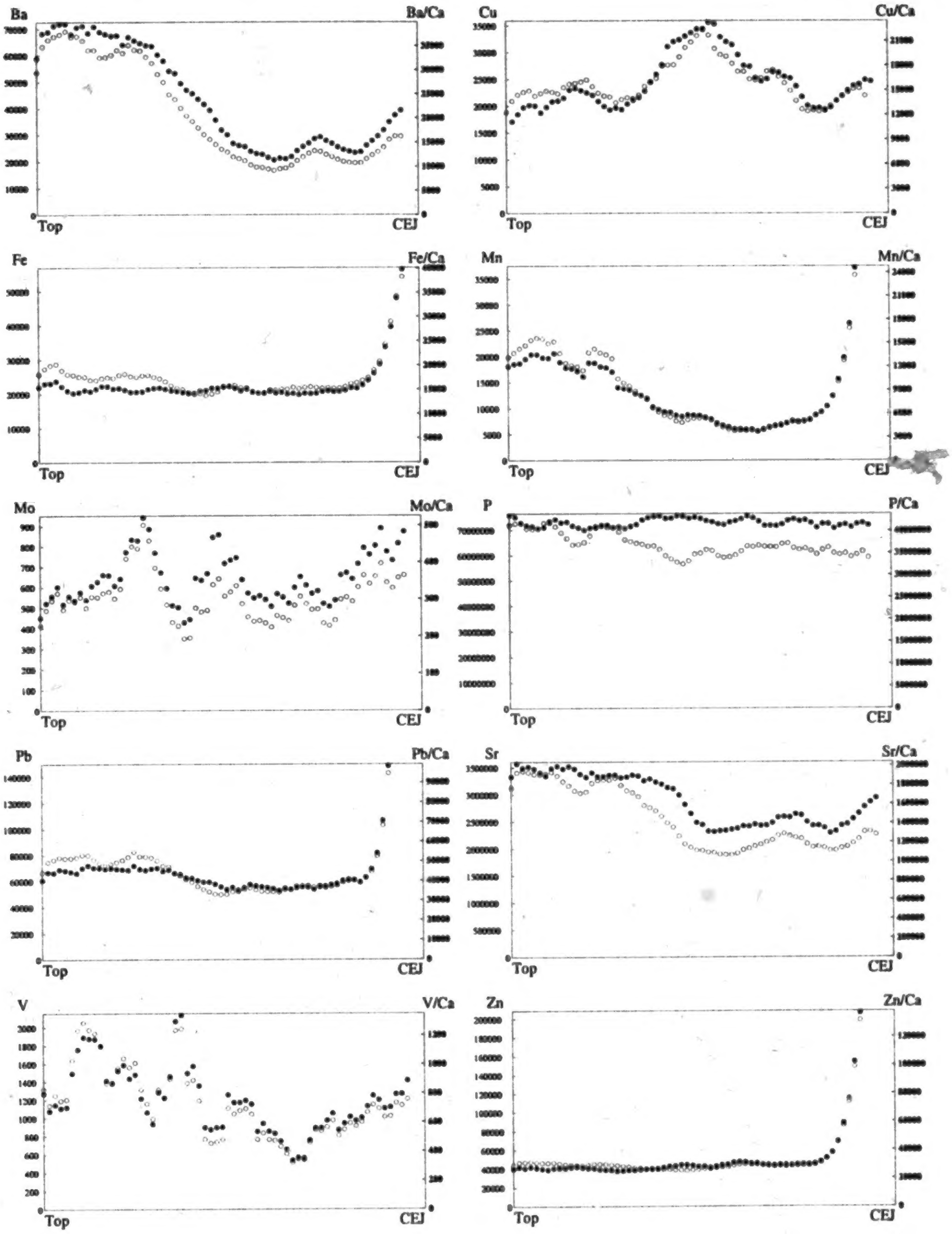


Fig. D-6: Element and element/Ca ratios for line d on the left third molar (P-LM3) of the maxilla. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

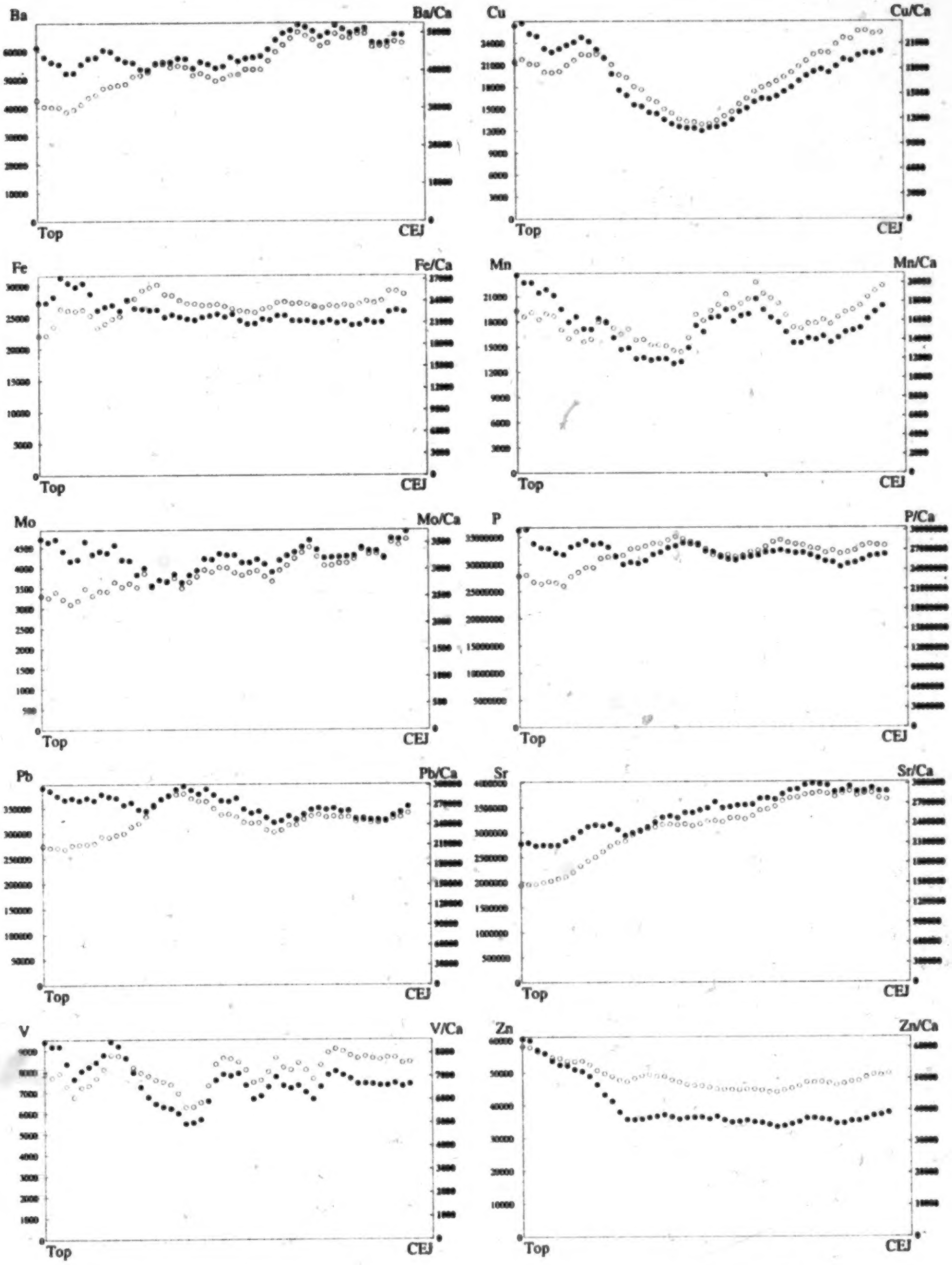


Fig. D-7: Element and element/Ca ratios for line d on the right first molar (P-RM1) of the maxilla. The line moves - from left to right - from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

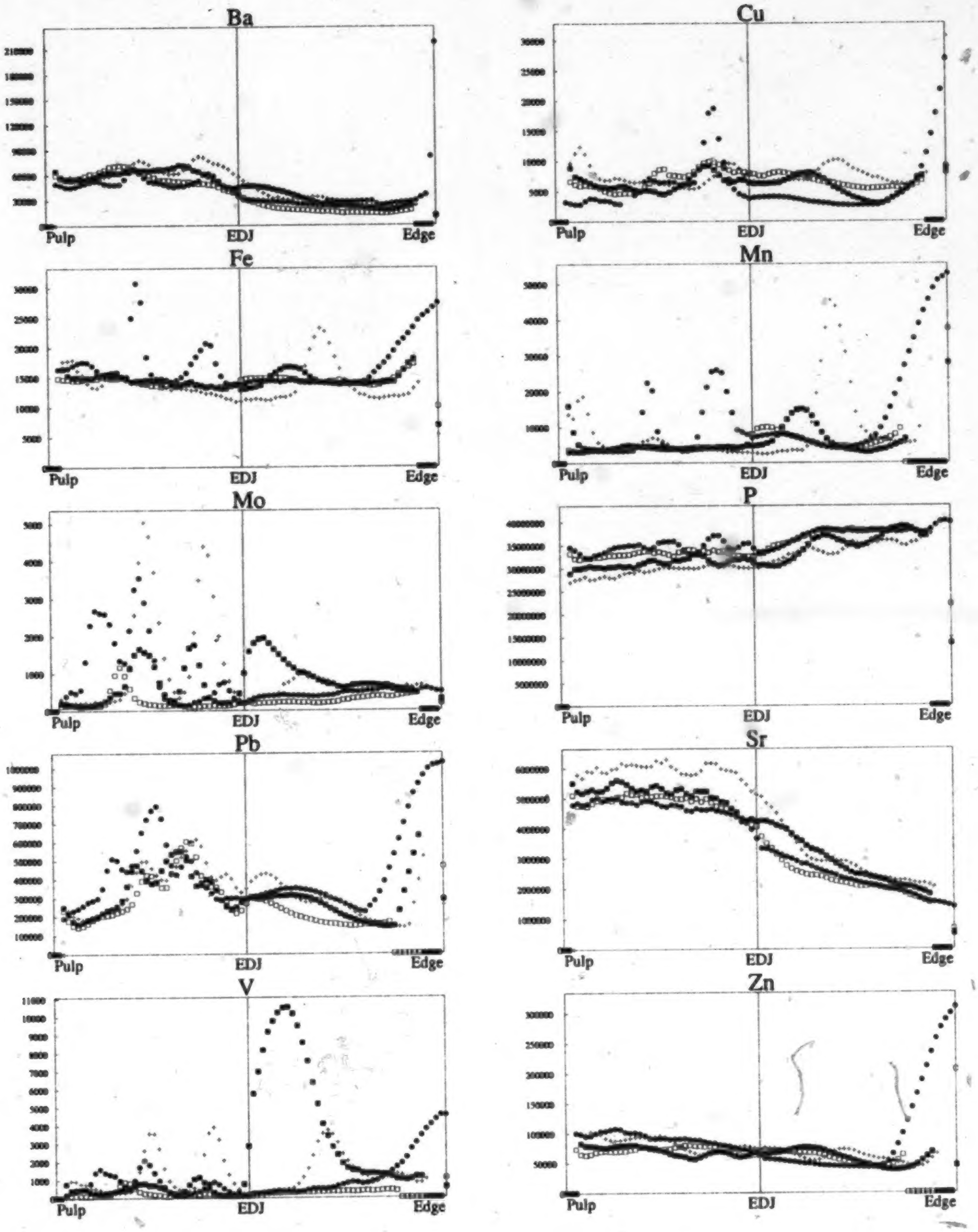


Fig. D-8: Summary graphs of laser tracks moving from inner dentine ('pulp') to outer enamel surface (edge) across the EDJ on the left first premolar (P-LP3). The lines represent smoothed data (removal of outliers and calculation of moving averages with a window of 9). + line a; ■ line c; □ line d; ● line e.

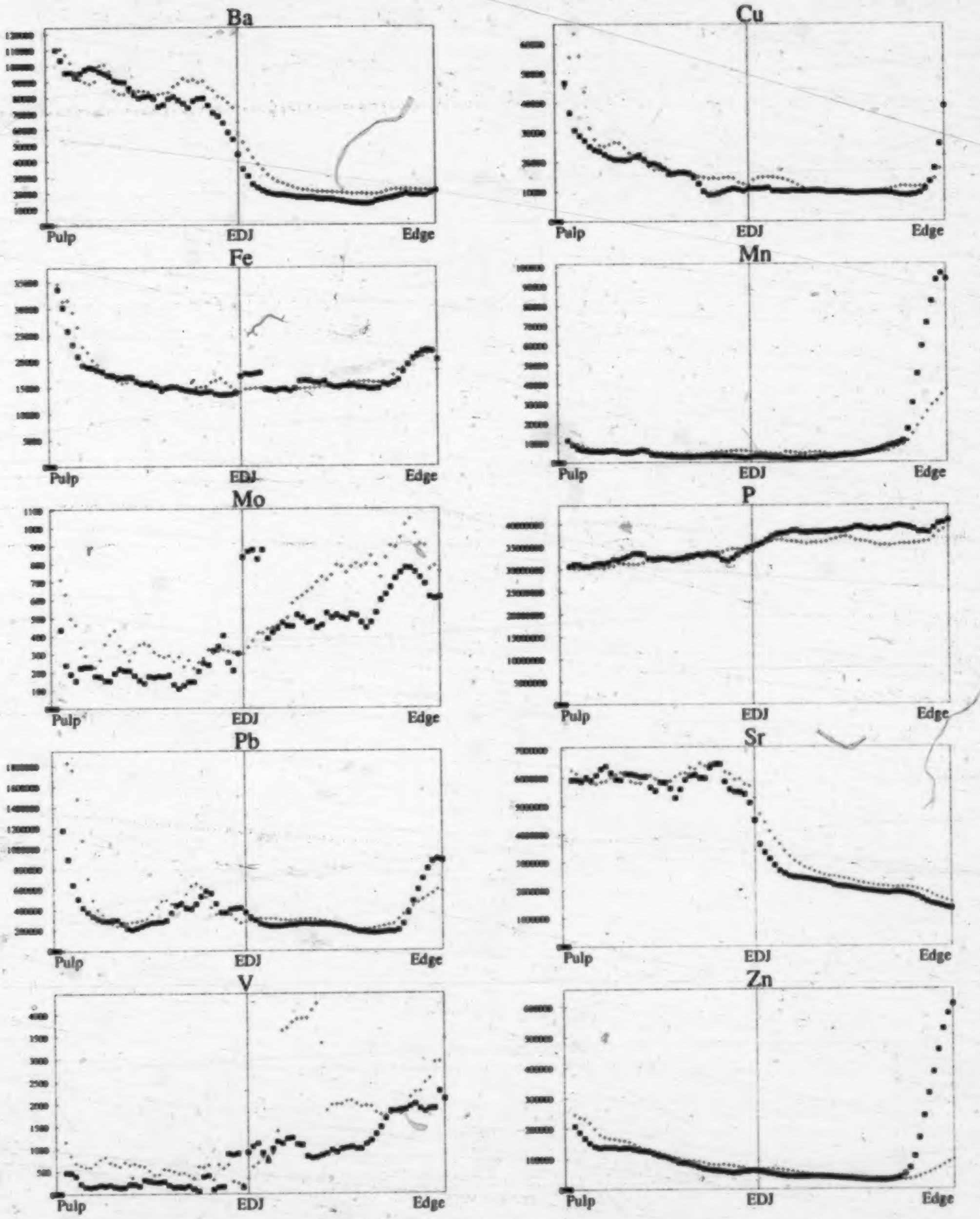


Fig. D-9: Summary graphs of laser tracks moving from inner dentine ('pulp') to outer enamel surface (edge) across the EDJ on the left second premolar (P-LP4). The lines represent smoothed data (removal of outliers and calculation of moving averages with a window of 9). + line a; ■ li[illegible] b.

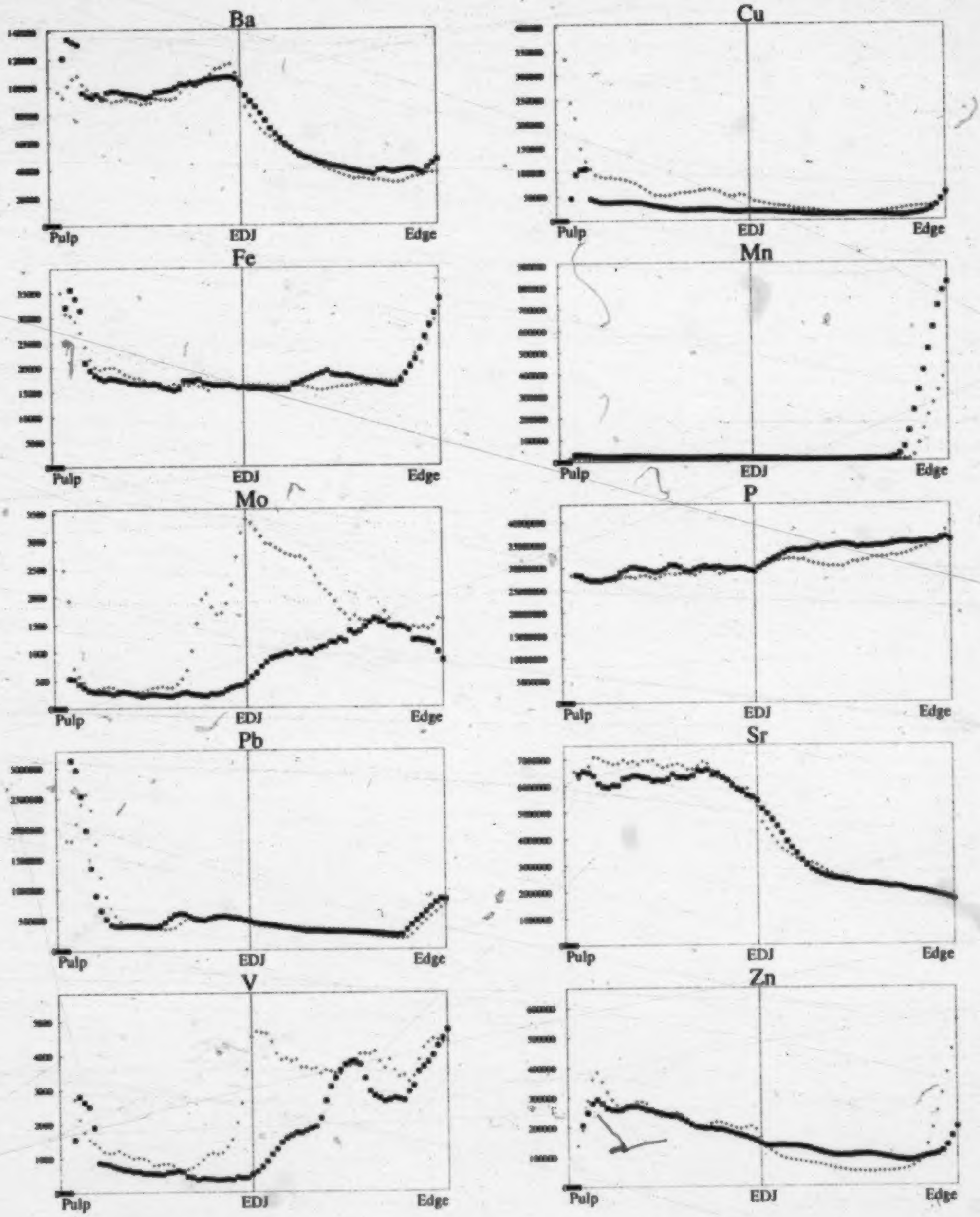


Fig. D-10: Summary graphs of laser tracks moving from inner dentine ('pulp') to outer enamel surface (edge) across the EDJ on the left first molar (P-LM1). The lines represent smoothed data (removal of outliers and calculation of moving averages with a window of 9). + line a; ■ line b.

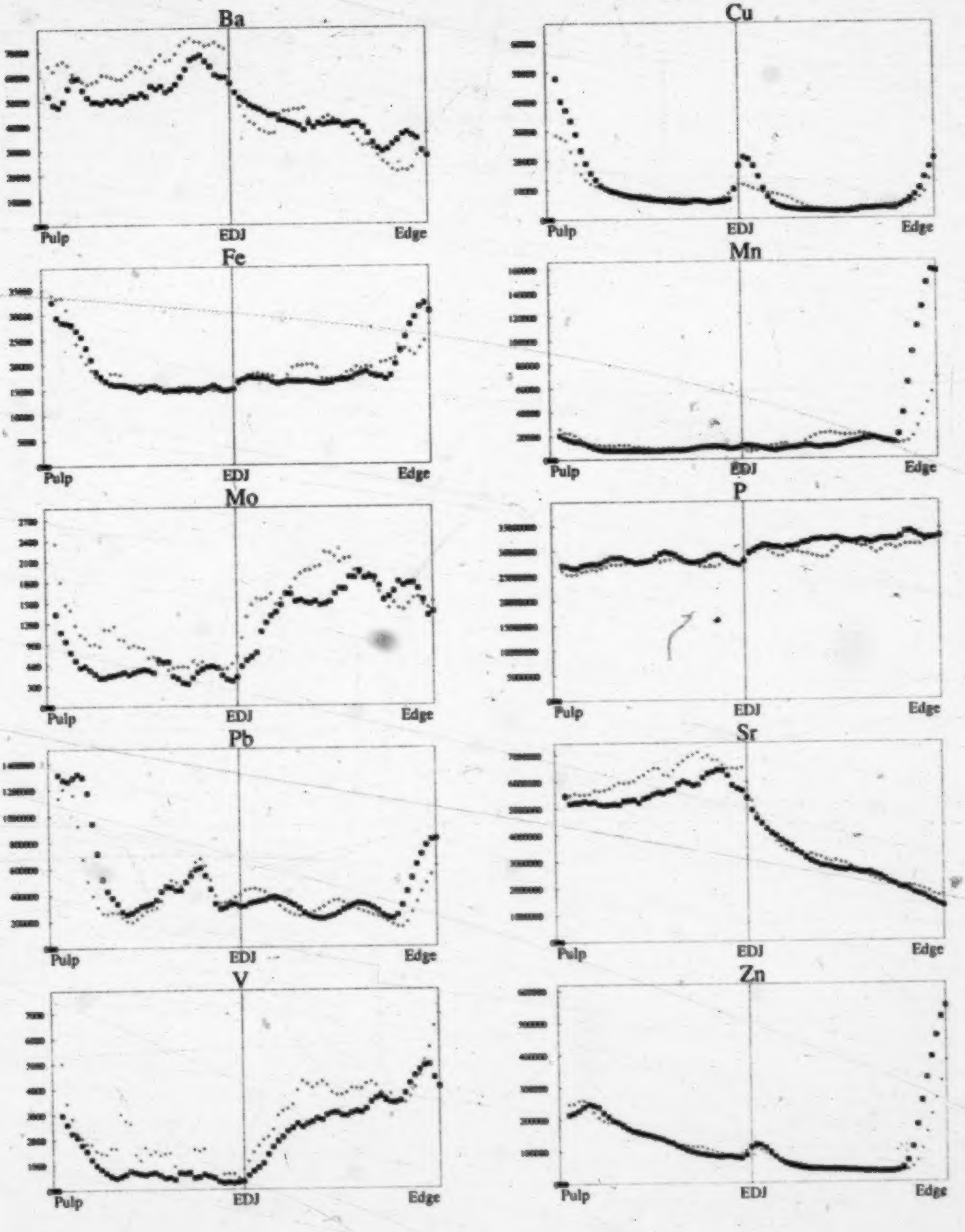


Fig. D-11: Summary graphs of laser tracks moving from inner dentine ('pulp') to outer enamel surface (edge) across the EDJ on the left second molar (P-LM2). The lines represent smoothed data (removal of outliers and calculation of moving averages with a window of 9). + line a; ■ line b.

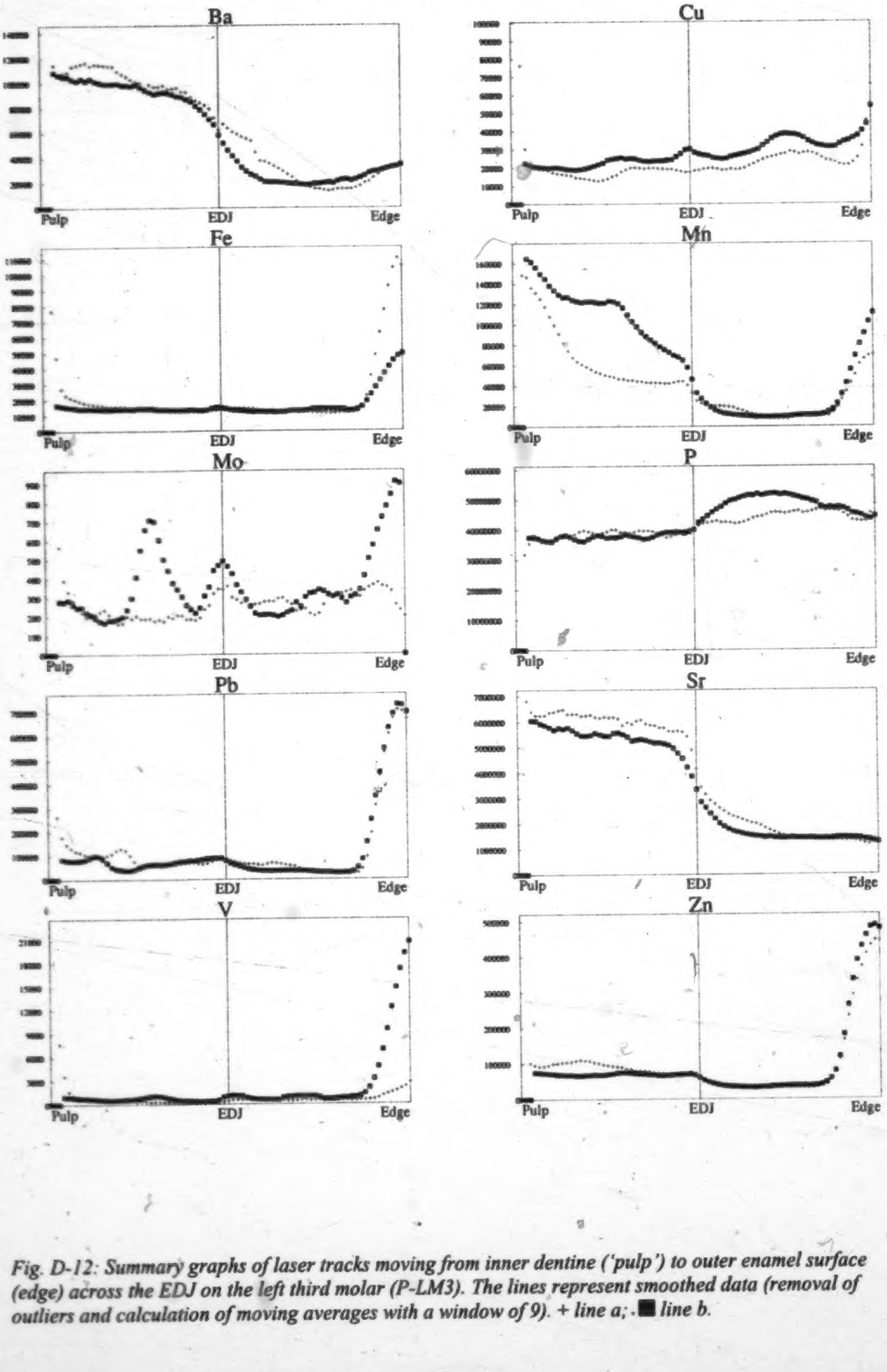


Fig. D-12: Summary graphs of laser tracks moving from inner dentine ('pulp') to outer enamel surface (edge) across the EDJ on the left third molar (P-LM3). The lines represent smoothed data (removal of outliers and calculation of moving averages with a window of 9). + line a; ■ line b.

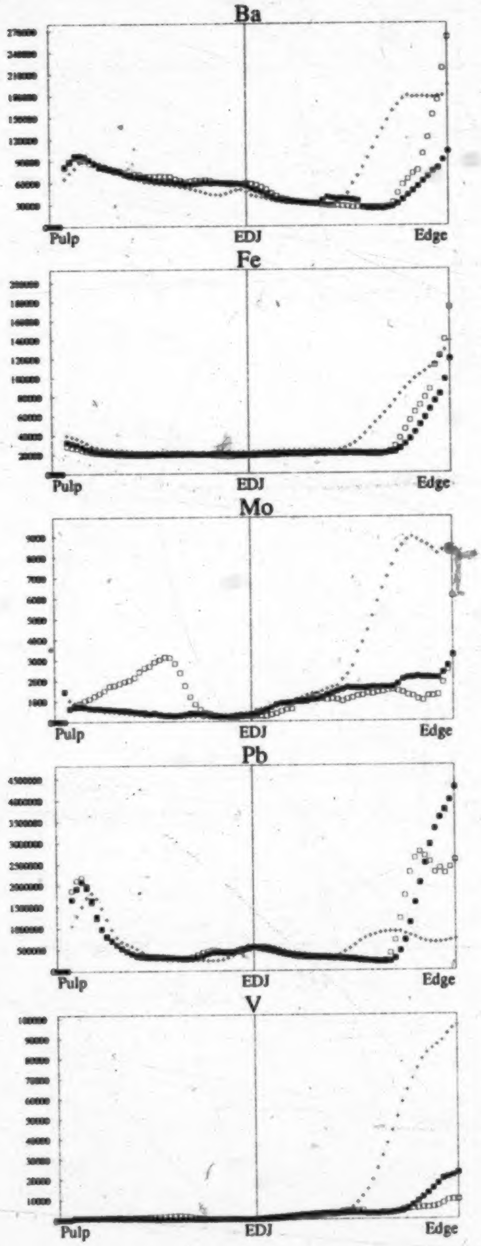
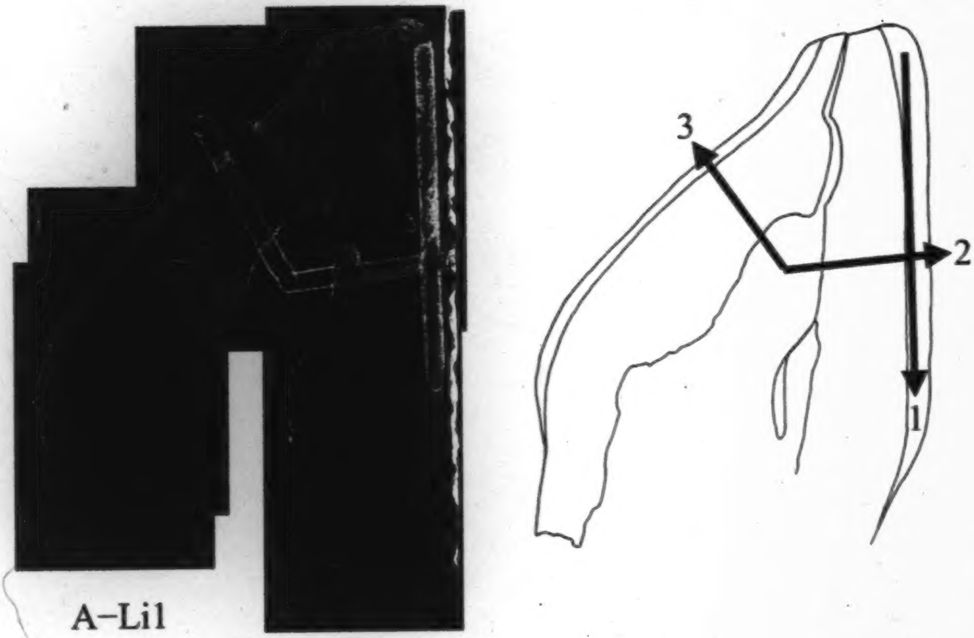


Fig. D-13: Summary graphs of laser tracks moving from inner dentine ('pulp') to outer enamel surface (edge) across the EDJ on the right first molar (P-RM1). The lines represent smoothed data (removal of outliers and calculation of moving averages with a window of 9). + line e; ■ line f; ☐ line g.

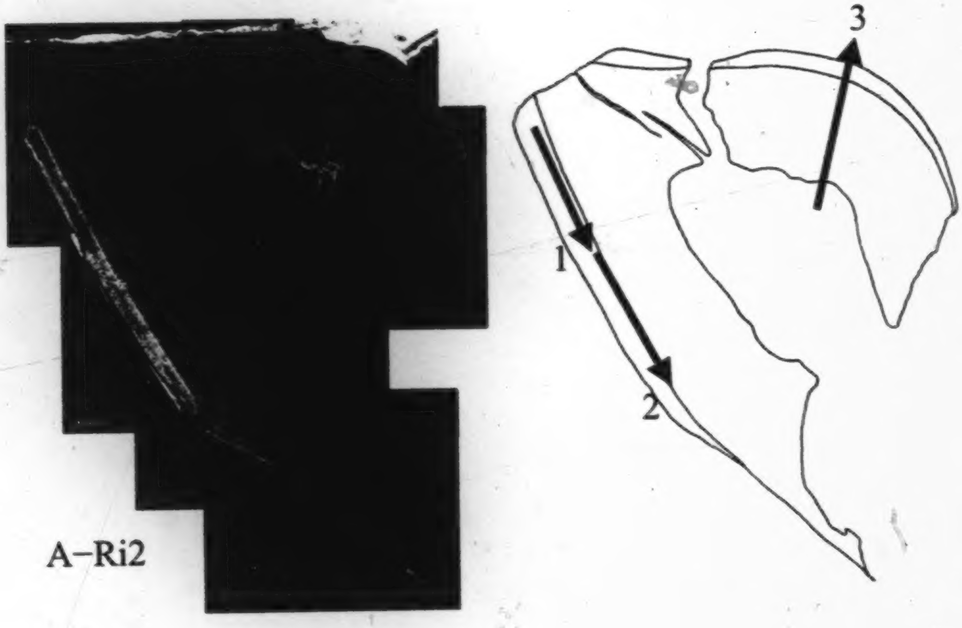
APPENDIX E: Laser Ablation ICP-MS (Deciduous teeth)

1. SEM-composites and line drawings based on these images, with arrows indicating the location and direction of the laser trajectories (pp. 241-248)

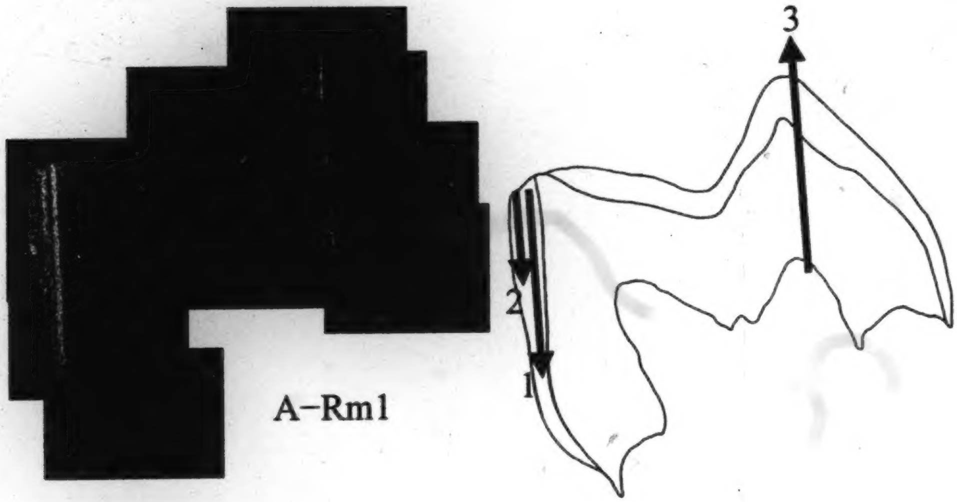
2. Plots showing the element and element/Ca ratios for the longitudinal lines on the deciduous teeth of individuals D, E and F. Figures E-1 to E-3 (pp. 249-251). **Cf. Figures 5.22-5.24.**



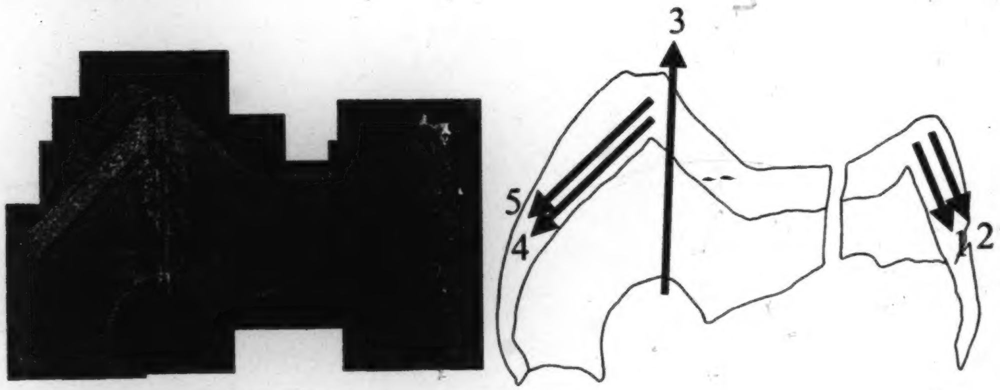
A–Li1



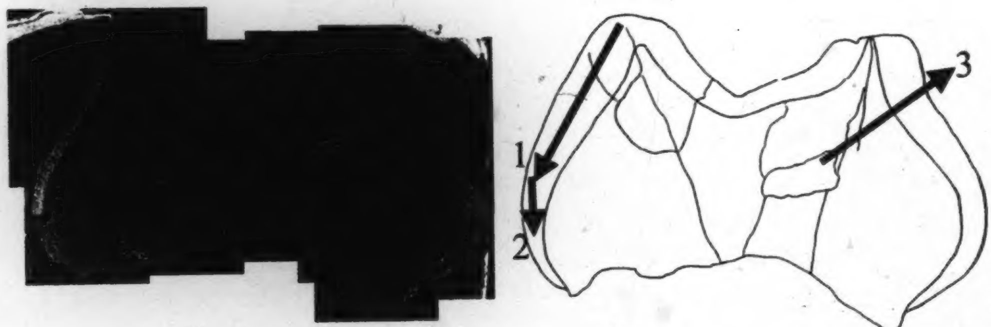
A–Ri2



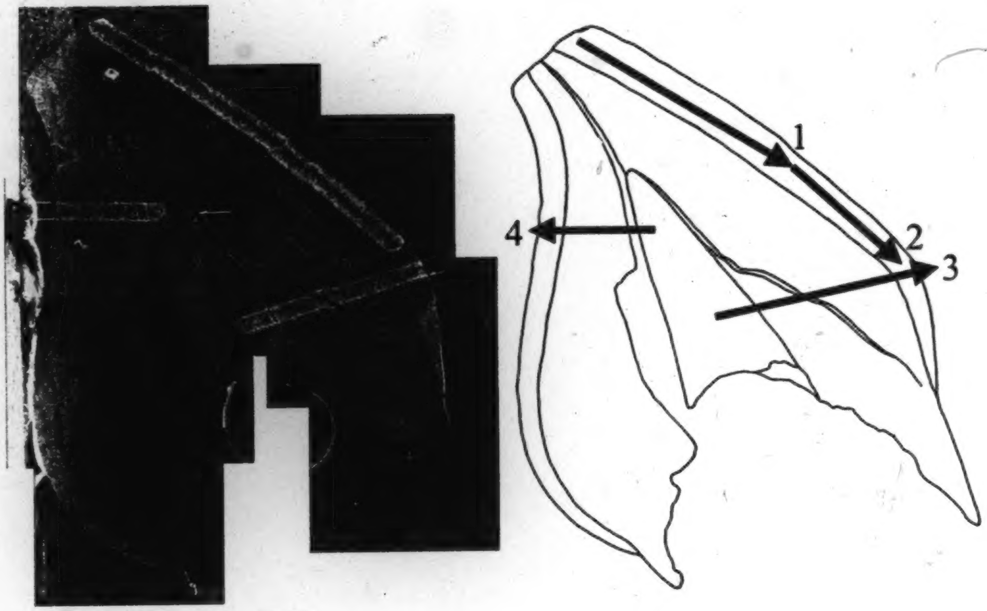
A−Rm1



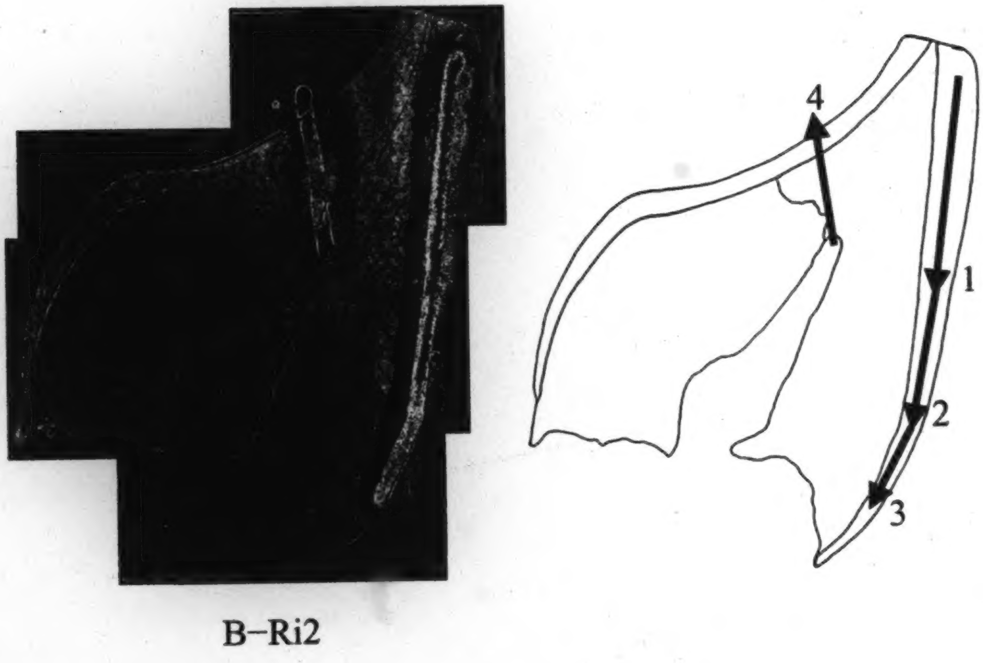
A−Rm2



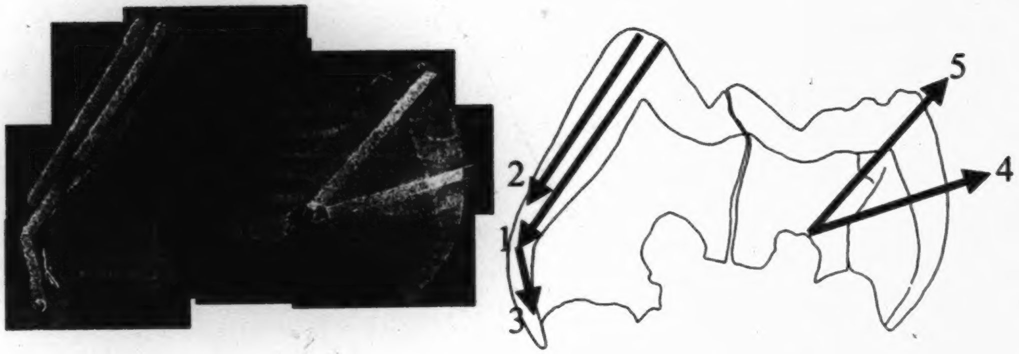
B−Rm1



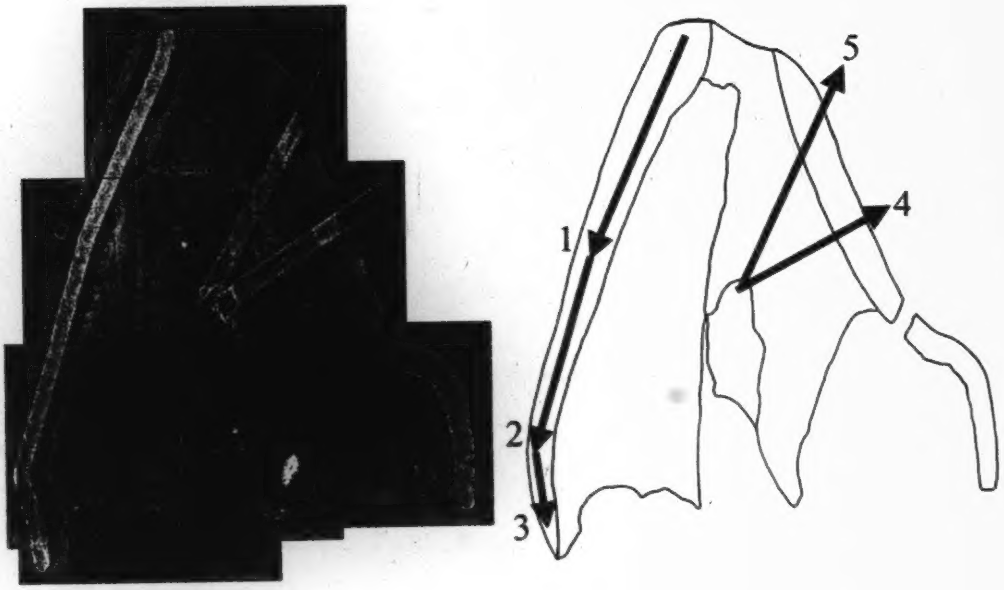
B–Ri1



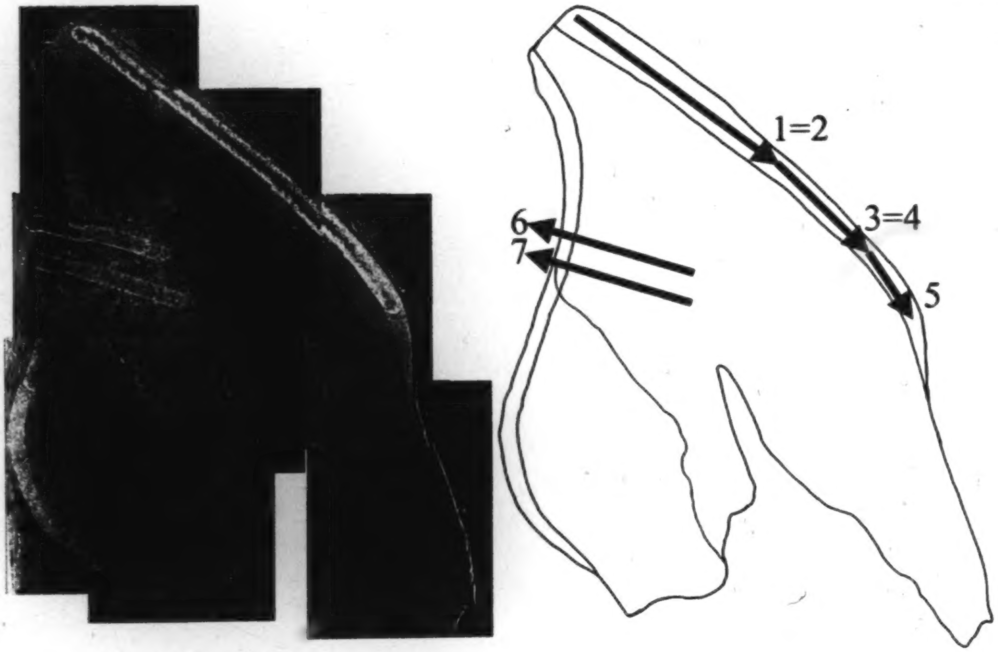
B–Ri2



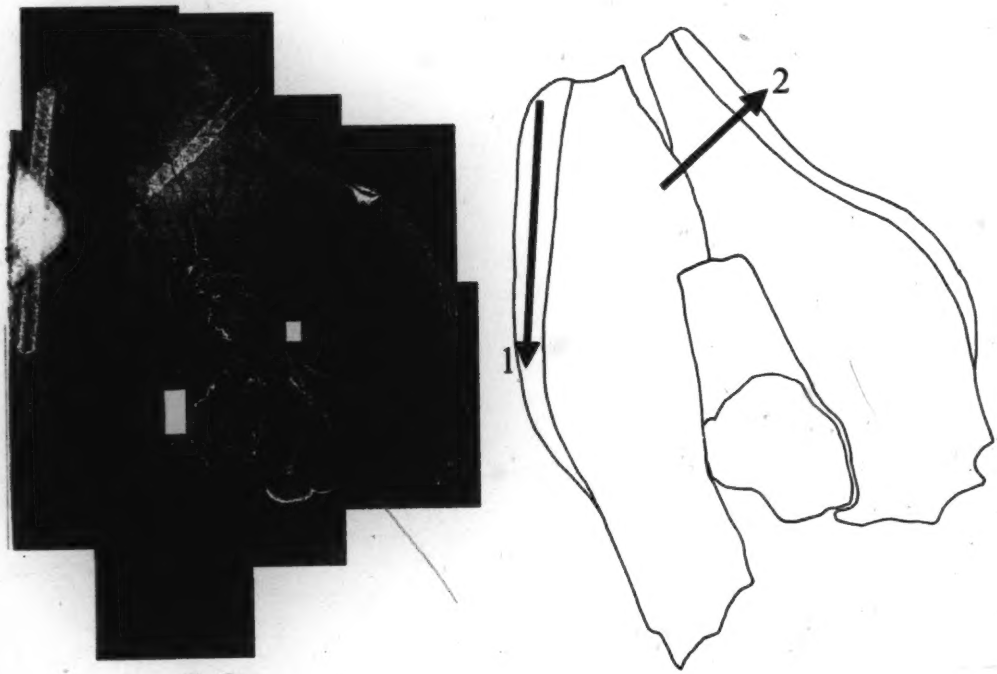
B–Rm2



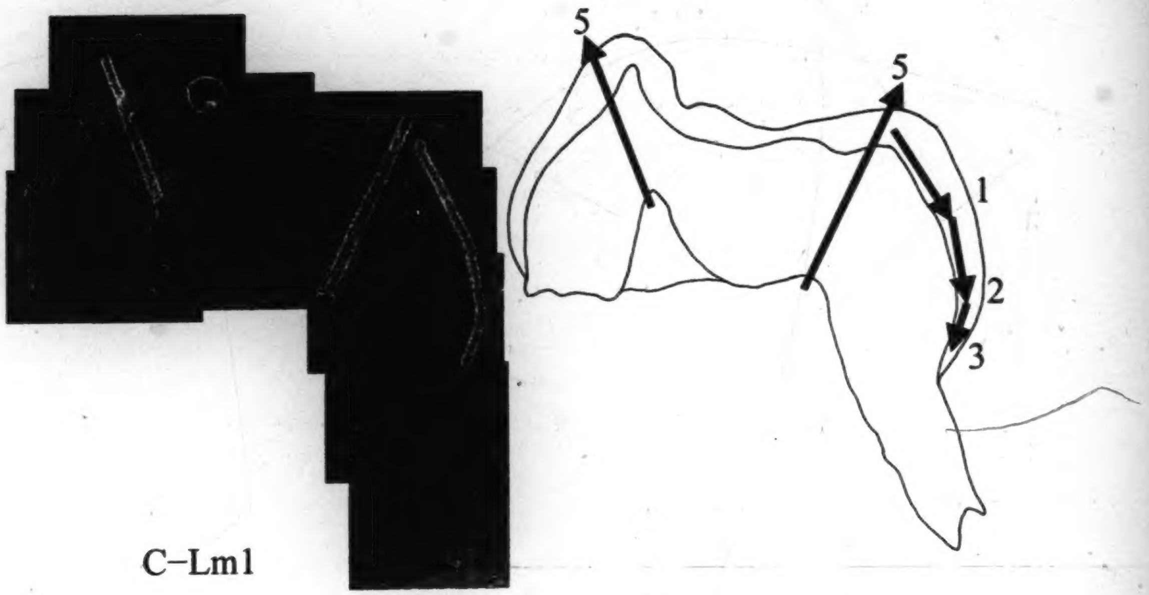
B–Rc



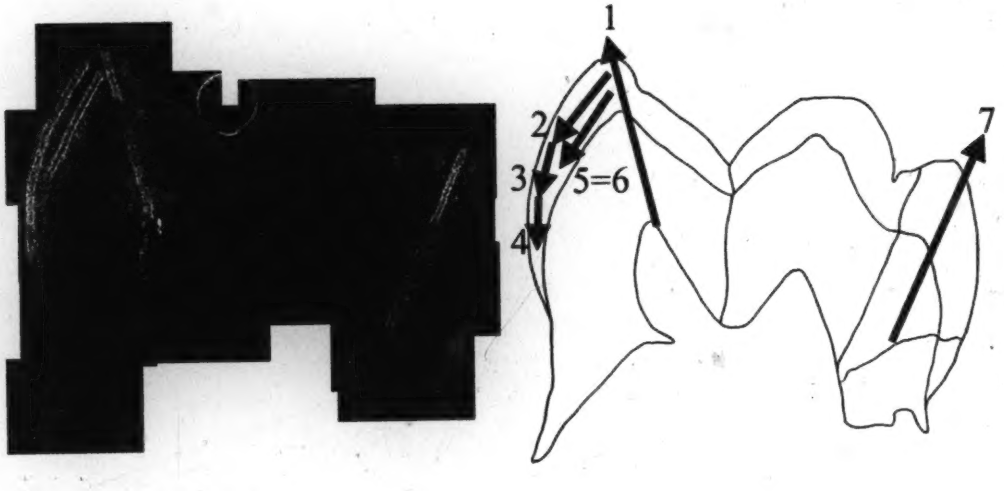
C−Ri1



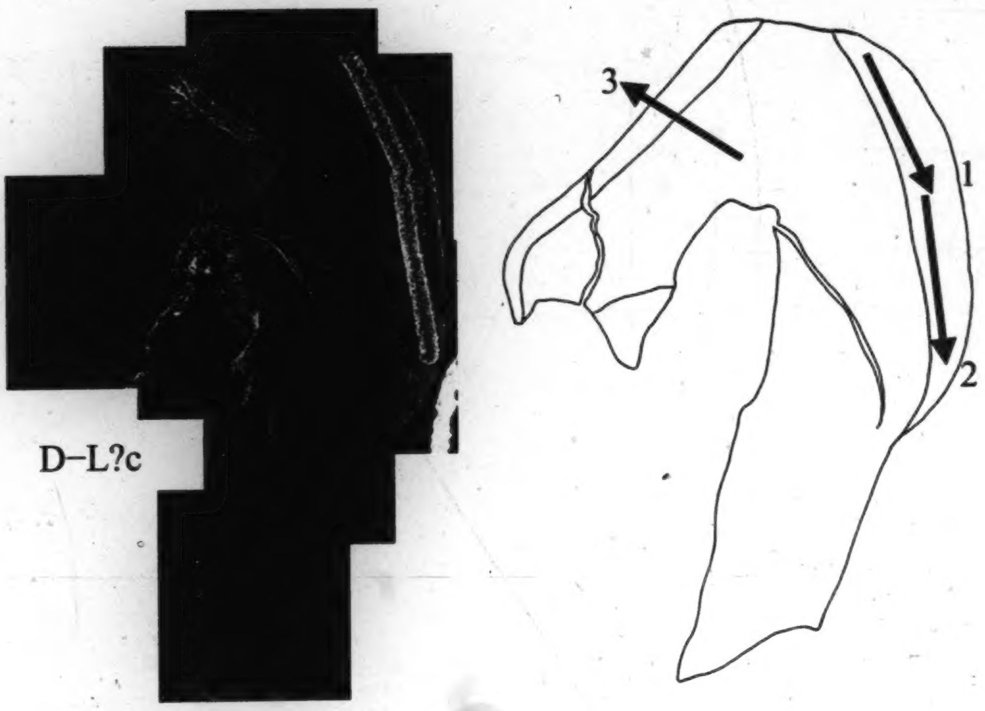
C−Lc



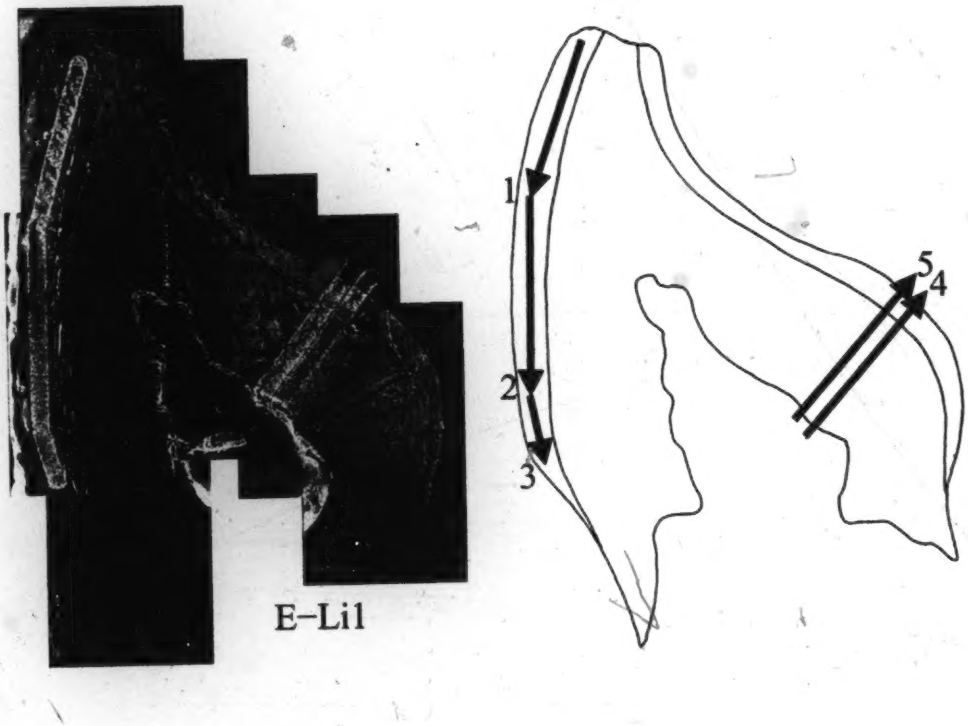
C−Lm1



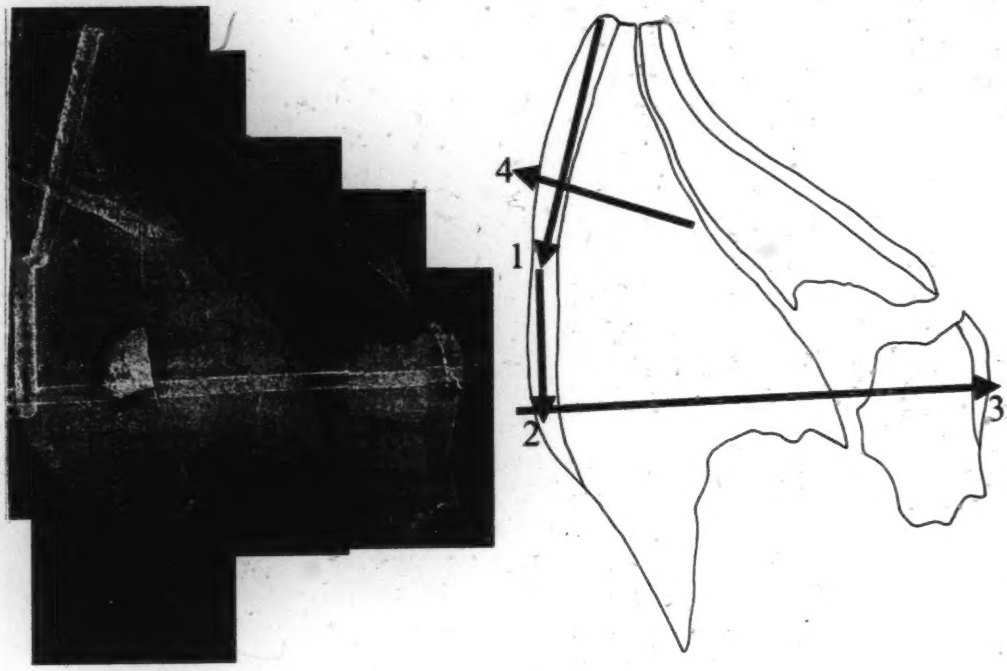
C−Lm2



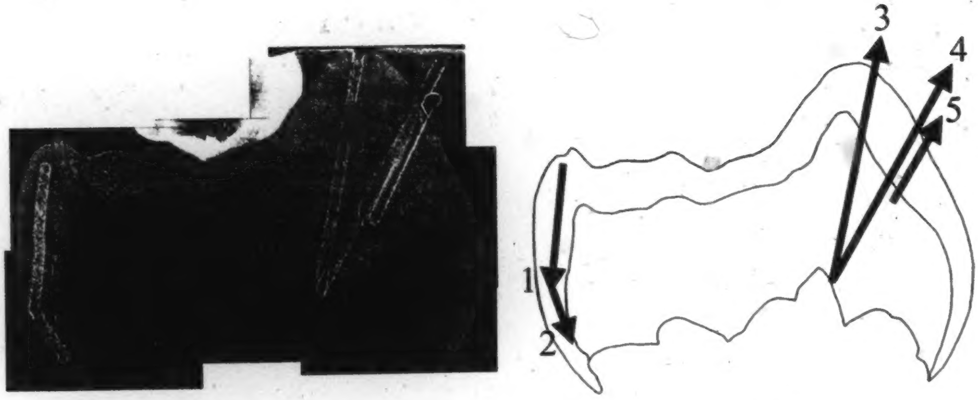
D−L?c



E−Li1



F−Li2



F−Lm2

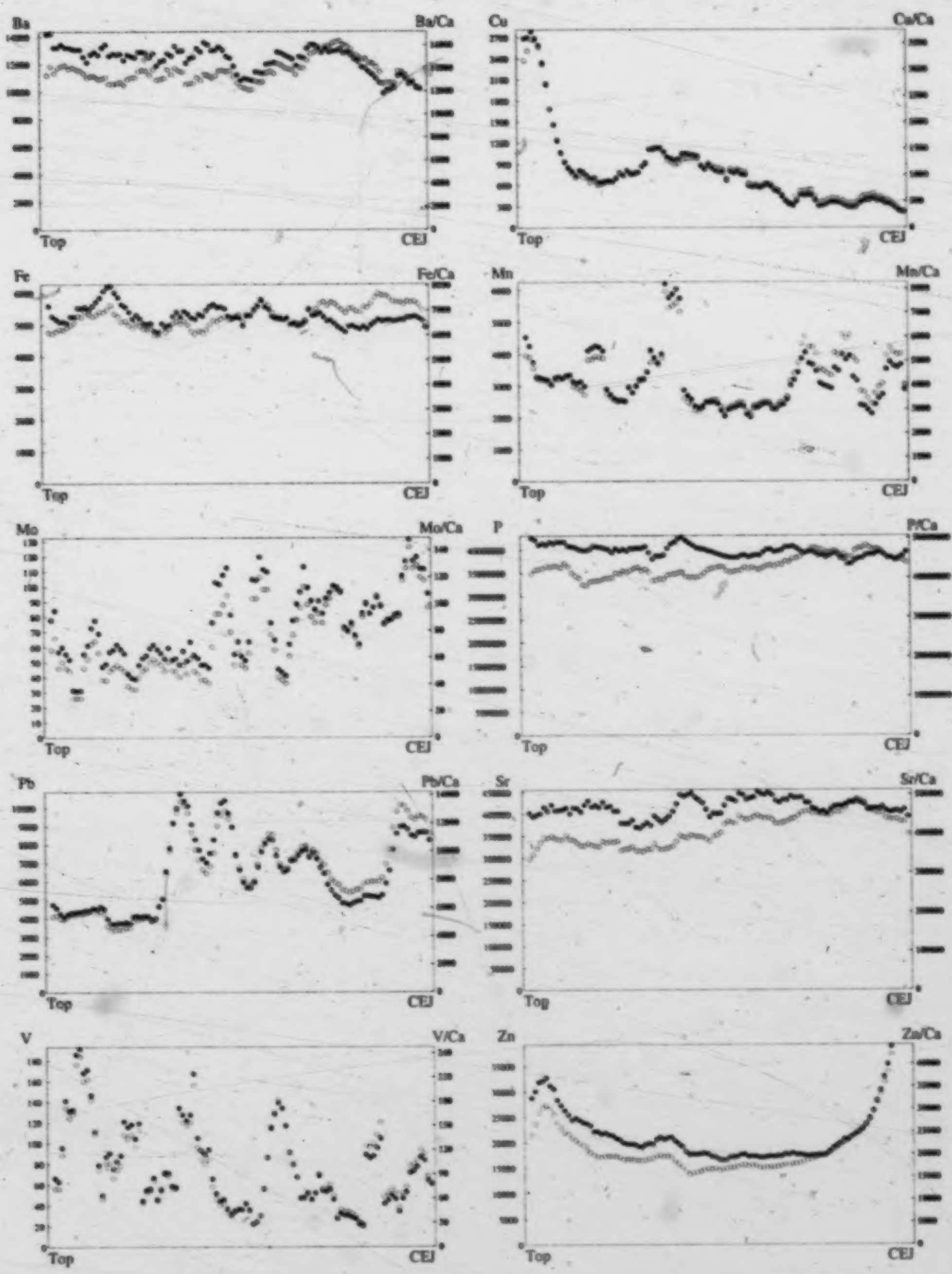


Fig. E-1: INDIVIDUAL D: Element and element/Ca ratios for combined longitudinal lines on the left canine (D-L?c). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

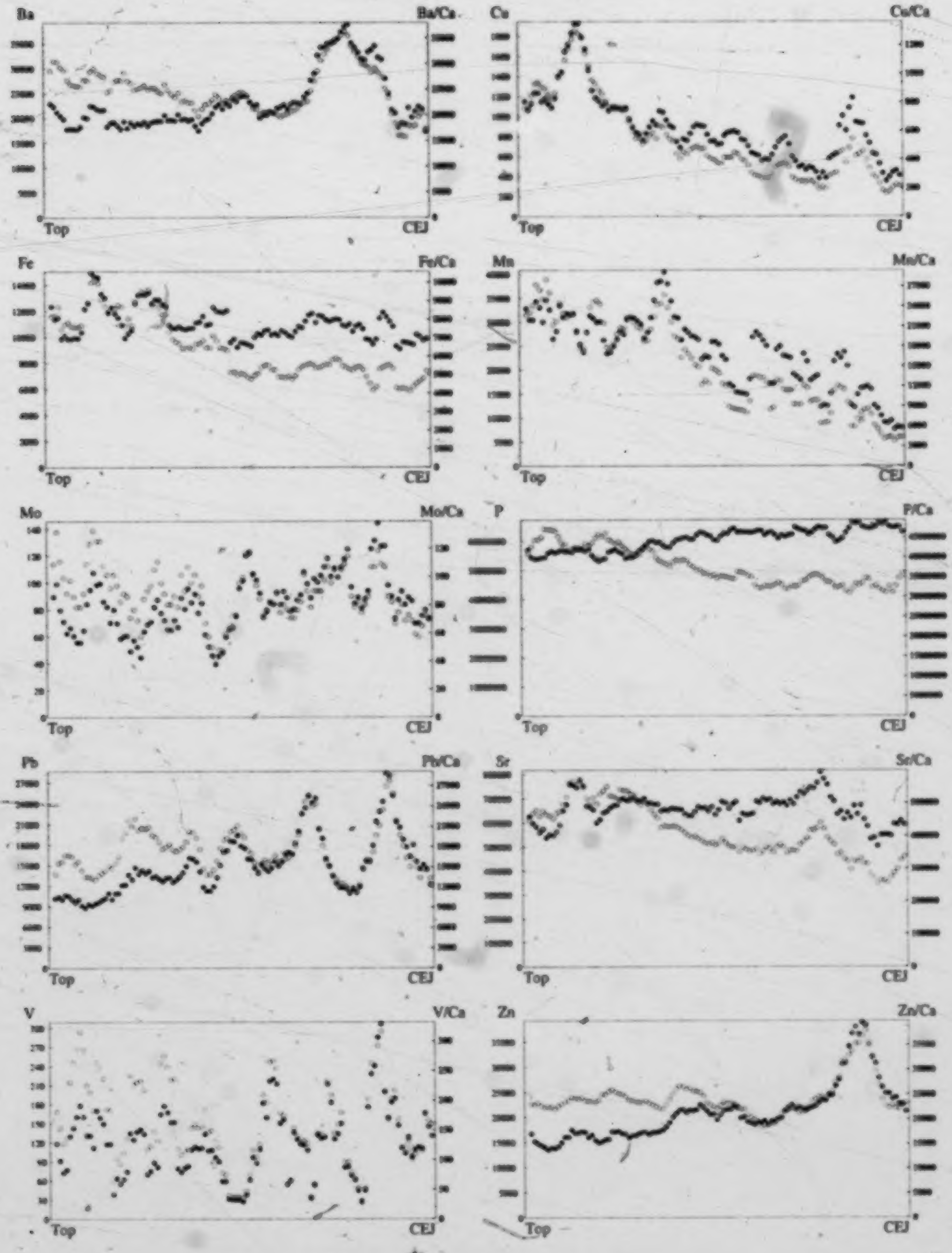


Fig. E-2: INDIVIDUAL E: Element and element/Ca ratios for combined longitudinal lines on the left central incisor(E-Li1). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).

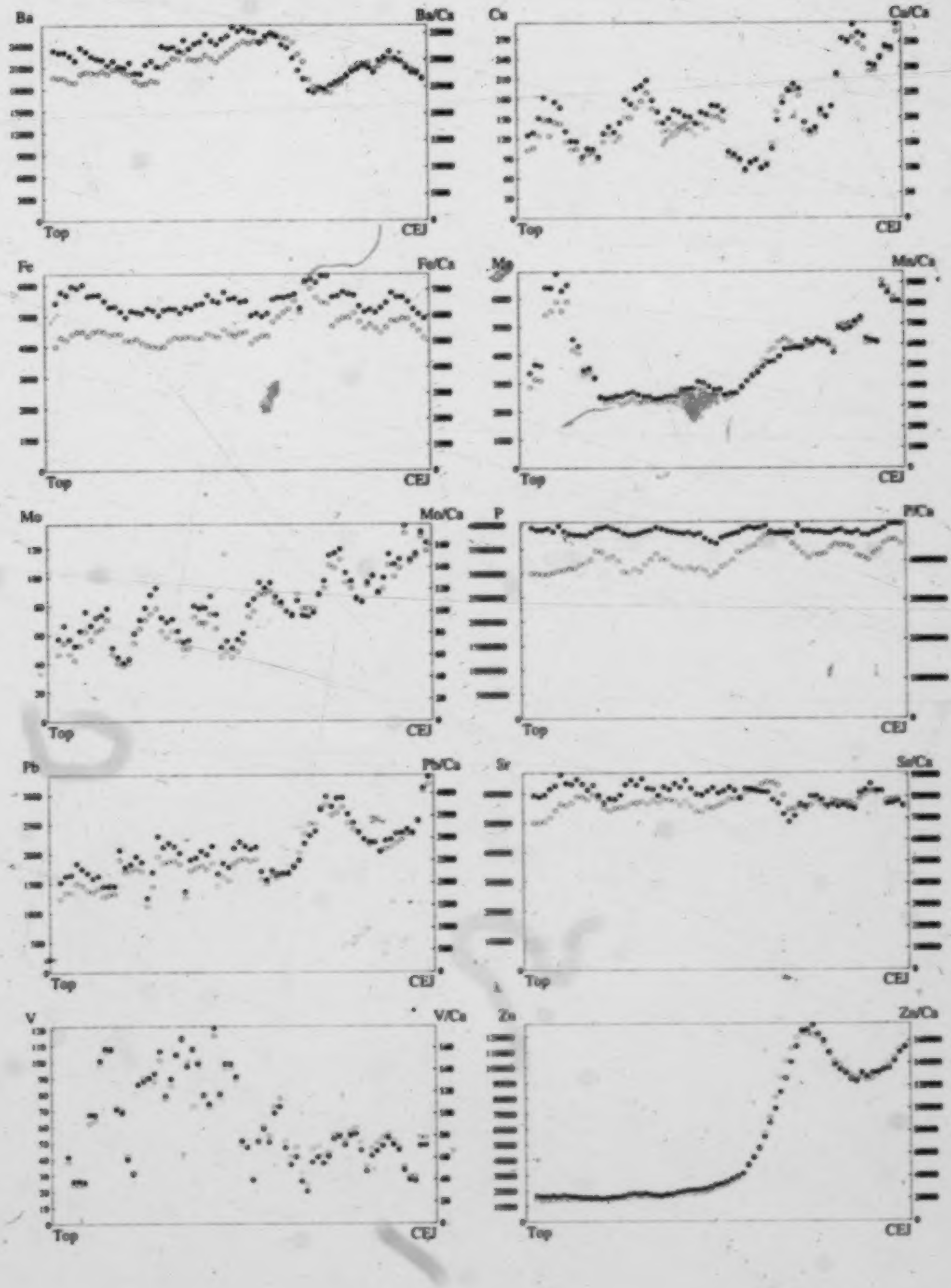


Fig. E-3: INDIVIDUAL F: Element and element/Ca ratios for combined longitudinal lines on the left second molar (F-Lm2). The line moves from the top of the crown towards the CEJ. Open circles = element (primary y axis); closed circles = element/Ca ratio (secondary y axis).